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REVIEW

Neuropeptide Y, the Hypothalamus, and Diabetes: Insights Into the Central Control of Metabolism

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FRANKISH, H. M., S. DRYDEN, D. HOPKINS, Q. WANG AND G. WILLIAMS. *Neuropeptide Y, the hypothalamus, and diabetes: Insights into the central control of metabolism.* PEPTIDES 16(4) 757–771, 1995.—Neuropeptide Y (NPY), a major brain neurotransmitter, is expressed in neurons of the hypothalamic arcuate nucleus (ARC) that project mainly to the paraventricular nucleus (PVN), an important site of NPY release. NPY synthesis in the ARC is thought to be regulated by several factors, notably insulin, which may exert an inhibitory action. The effects of NPY injected into the PVN and other sites include hyperphagia, reduced energy expenditure and enhanced weight gain, insulin secretion, and stimulation of corticotropin and corticosterone release. The ARC-PVN projection appears to be overactive in insulin-deficient diabetic rats, and could contribute to the compensatory hyperphagia and reduced energy expenditure, and pituitary dysfunction found in these animals; overactivity of these NPY neurons may be due to reduction of insulin's normal inhibitory effect. The ARC-PVN projection is also stimulated in rat models of obesity ± non-insulin diabetes, possibly because the hypothalamus is resistant to inhibition by insulin; in these animals, enhanced activity of ARC NPY neurons could cause hyperphagia, reduced energy expenditure, and obesity, and perhaps contribute to hyperinsulinemia and altered pituitary secretion. Overall, these findings suggest that NPY released in the hypothalamus, especially from the ARC-PVN projection, plays a key role in the hypothalamic regulation of energy balance and metabolism. NPY is also found in the human hypothalamus. Its roles (if any) in human homeostasis and glucoregulation remain enigmatic, but the animal studies have identified it as a potential target for new drugs to treat obesity and perhaps NIDDM.

Neuropeptide Y Insulin Hypothalamus Diabetes Obesity Rat

THIS review will focus on the possible role of a brain peptide, neuropeptide Y (NPY), in mediating some of the hypothalamic disturbances that occur in diabetic rodents. At first sight, this may seem a very restricted brief: why have we selected only one of the 40 or so neurotransmitters found in the hypothalamus, and why are we concentrating on diabetes? The immediate answers to these questions are that NPY is now convincingly implicated in regulating important hypothalamic functions, and the changes that accompany diabetes may provide valuable insights into the central control of activities such as energy homeostasis, metabolism, and pituitary secretion.

Diabetes itself demands attention because it is common, causes considerable morbidity and mortality, and imposes immense financial burdens on society. Worldwide, diabetes may affect over 50 million people and in the USA and other developed countries, it probably absorbs 5–10% of total health-care expenditure. The most obvious problems posed by diabetes relate to its long-term management and its chronic complications, but certain hypothalamic disturbances associated with diabetes may be clinically significant in some cases. Examples of neuroendocrine dysfunction include disturbed secretion of pituitary hormones, notably growth hormone and cortisol, which, by impairing tissue sensitivity to insulin, may contribute to poor metabolic control in some patients. This may apply particularly to pubertal patients, and the delayed insulin resistance caused by nocturnal hypersecretion of growth hormone has been held responsible for the fasting hyperglycemia of the "dawn phenomenon." Disordered gonadotropin secretion may lead to menstrual irregularity and reduced fertility, although, like all aspects of pituitary dysfunction in human diabetes, this tends to improve with tightened glycemic control and therefore poses less of a clinical problem nowadays than previously.

Perhaps the most important aspect, however, is the role that the hypothalamus may play in causing obesity, and therefore in contributing to non-insulin-dependent diabetes (NIDDM). Most people with NIDDM are obese, and failure to lose weight is a common and significant barrier to effective treatment in many cases (143). NIDDM accounts for over 80% of diabetes in Eu-

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rope and North America; apart from the obvious intellectual rewards, a deeper understanding of how energy balance is controlled could potentially improve the management of over 50% of the diabetic people in the Westernized world.

We shall first discuss briefly the various diabetic syndromes of man and rodents, and the nature and significance of the hypothalamic disturbances that accompany them. Second, we shall discuss NPY, its experimental actions and the factors that may regulate the activity of NPYergic pathways in the hypothalamus. Third, we shall review critically the evidence that NPY may be involved in causing some of the neuroendocrine changes of diabetes. Finally, we shall try to determine whether the lessons learned from animal studies are likely to survive the ascent of the evolutionary ladder, and whether they have any relevance to diabetes and nutritional disorders in man.

DIABETIC SYNDROMES IN MAN AND RODENTS

Human insulin-dependent diabetes mellitus (IDDM) is due to chronic autoimmune destruction of the pancreatic β-cells. This may smoulder unsuspected for many years until a critical mass (probably >90%) of the β-cells has been destroyed. At this stage, blood glucose levels rise and the clinical features of the disease (polyuria, polydipsia, weight loss, malaise) appear relatively rapidly. Insulin deficiency in IDDM is so profound that unrestrained lipolysis and ultimately ketosis develop, in addition to hyperglycemia. Patients with IDDM die unless insulin replacement is given.

Several diabetic syndromes in rodents resemble human IDDM quite faithfully. The BB (Bio-Breeding) rat and NOD (non-obese diabetic) mouse both develop spontaneous autoimmune insulitis, analogous to that in man, leading to profound insulin deficiency, severe hyperglycemia, ketosis, and dependence on insulin replacement for survival. A more convenient and readily controlled model is that induced in normal rats by administering the β-cell toxin, streptozotocin (STZ), a *Streptomyces*-derived compound whose potential use as an antibiotic was abandoned when its diabetogenic properties became apparent. High doses of STZ (>100 mg/kg) cause severe insulin deficiency, ketosis, and death, whereas a single dose of 50–100 mg/kg induces a relatively stable IDDM-like syndrome. Plasma insulin concentrations are reduced to 10–20% of normal, glycemia runs at 25–30 mmol/l, and the rats fail to gain weight; at this dosage, ketosis does not develop and they can survive for prolonged periods without insulin (14).

Human NIDDM is due to a variable combination of insulin deficiency, which is less severe than in IDDM, together with reduced ability of the target tissues to respond to insulin action (i.e., insulin insensitivity or insulin resistance). Highly specific two-site immunoradiometric assays, which recognize only authentic insulin, have recently shown beyond doubt that circulating insulin concentrations are subnormal in NIDDM, whereas conventional assays (which also detect incompletely processed insulin precursors) have yielded inconsistent findings. Longitudinal studies of pre-NIDDM subjects suggest that insulin resistance precedes and may hasten β-cell failure. Obesity is a major determinant of insulin resistance and is undoubtedly of etiologic importance in many cases of NIDDM (153).

NIDDM-like states can be induced in rats by partial pancreatectomy or small doses of STZ (≈10 mg/kg) given during the neonatal period; both procedures result in moderate insulin deficiency without ketosis (15,106). Various spontaneous models of NIDDM have also been reported, notably two genetic syndromes of mice, which are both inherited as autosomal recessive traits. These are the *ob/ob* and *db/db* mice, which are homozygous for the *ob* (obese) and *db* (diabetes) gene, respectively. As the name implies, *ob/ob* mice are obese, weighing about twice as much as their unaffected (+/+ or +/*ob*) littermates. They are insulin resistant and have marked hyperinsulinemia: plasma insulin concentrations, measured by conventional RIAs, are 10–20 times higher than in lean mice. Blood glucose levels are up to 20–30 mmol/l. *db/db* mice are similar, but their insulin secretion is more precarious and falls in later life, leading to weight loss, severe hyperglycemia, ketosis, and insulin dependence (8).

In rats, homozygosity for the autosomal recessive genes, *fa* (fatty) and *cp* (corpulent) also results in obesity, insulin resistance, and hyperinsulinemia. The *fa/fa* syndrome was first identified in the Zucker strain, but can also be transferred to other strains such as the Wistar (56). The *cp/cp* syndrome has been expressed on various backgrounds, including the LA-N and the JCR:LA. All these mutants display spectacular obesity, and some individuals weigh over 1 kg. Insulin resistance is severe, with skeletal muscle being particularly insensitive to insulin action, and plasma insulin concentrations may be increased up to 10–20 times normal (8). Some syndromes, notably the JCR:LA-*cp/cp*, also show severe lipid abnormalities with accelerated atherosclerosis, and die prematurely from myocardial infarction. These animals have been equated with Syndrome X in man (comprising insulin resistance, hyperinsulinemia, glucose intolerance, dyslipidemia, and hypertension), which is now thought to be a major risk factor for coronary heart disease. However, these insulin-resistant syndromes correspond poorly to human NIDDM because they are only mildly glucose intolerant and display modest hyperglycemia that falls short of the diagnostic criteria for diabetes. Recently, introduction of the *fa* gene into the Wistar rat (56) has produced an obesity/hyperinsulinemia syndrome with greater hyperglycemia (>22 mmol/l), and a variant of the *fa/fa* Zucker, the Zucker diabetic fatty (ZDF) that becomes markedly hyperglycemic, appears to resemble human NIDDM even more closely. Further characterization of these models is awaited and, in particular, definition of the extent to which "true" insulin may contribute to their hyperinsulinemia.

NEUROENDOCRINE DISTURBANCES IN DIABETIC RODENTS

Many facets of neuroendocrine function are disturbed, in different ways, in the rodent models of IDDM and NIDDM (Table 1).

TABLE 1

NEUROENDOCRINE AND METABOLIC DISTURBANCES IN ANIMAL MODELS OF IDDM/NIDDM

	Insulin-Deficient Diabetes	Obese-Hyperinsulinemic Diabetes
Energy metabolism		
Food intake	increased	increased
Energy expenditure	decreased	decreased
Body weight	decreased	increased
Blood insulin	decreased	increased
Pituitary secretion		
Blood corticosterone	increased	increased
Gonadotropins	decreased	decreased
Growth hormone	decreased	normal
Prolactin	decreased	normal
Thyrotropin	decreased	normal
Other activities		
Circadian rhythms	disturbed	disturbed
Sexual activity	reduced	reduced

IDDM-like syndromes display striking changes in energy metabolism, pituitary secretion, circadian rhythms, and in certain behaviors. An obvious alteration in energy metabolism is hyperphagia; food intake rises by 50–100% within a few days of inducing experimental diabetes in rats, with a marked preference for carbohydrate-rich foods (65). The basis for the carbohydrate selectivity is not clear but it does not appear to be determined by taste alone. Energy expenditure is reduced, and the thermogenic capacity of brown adipose tissue (measured as mitochondrial GDP binding or its content of uncoupling protein mRNA) is decreased. These responses may represent adaptive attempts to maintain energy balance in the face of diabetes, which is an intensely catabolic state that also leads to considerable losses of energy substrate through glycosuria.

The secretion of all pituitary hormones is abnormal to some extent, and may be accompanied by histologic changes in the relevant pituitary cell types and by secondary alterations in the endocrine organs that they regulate. Secretion of corticosterone is increased (101), with loss of the normal diurnal secretory rhythm, as is seen with cortisol in human Cushing's disease. Growth hormone secretion is decreased (128), the opposite to the change seen in human IDDM. Prolactin release is also reduced (63). The secretion of gonadotropins, especially of LH, is impaired (64) and this has been implicated in the high frequency of anovulatory cycles and reduced fertility in untreated female diabetic rats. Finally, thyrotropin secretion is decreased (49), leading to secondary hypothyroidism. Behavioral changes include an increase in food-seeking activity as well as hyperphagia, together with corresponding decreases in sexual and reproductive behavior. Drinking is also increased, and water intake may be 10 times higher than in nondiabetic rats.

In the genetic syndromes of obesity with glucose intolerance or NIDDM, obesity is due primarily to a reduction in energy expenditure rather than to increased energy intake. Reduced energy expenditure appears to result from defective stimulation of thermogenesis in brown adipose tissue (BAT) by the sympathetic nervous outflow from the CNS, rather than to an intrinsic abnormality of BAT itself. All of these animals are hyperphagic compared with their nonobese counterparts, for at least part of their lifespan, and this undoubtedly exacerbates obesity. However, the overriding importance of reduced energy expenditure in these syndromes is demonstrated by the fact that obesity still develops if food intake is not allowed to rise above that of their lean counterparts (26), and recently, by the elegant demonstration that transgenic mice whose BAT has been ablated also become obese (80). Another defect in these syndromes is hyperinsulinemia, which appears early and may precede the appearance of significant obesity. This may also result from central (hypothalamic) dysregulation of autonomic function, with dominance of the parasympathetic outflow that stimulates insulin secretion, over the inhibitory sympathetic system (72). Pituitary dysfunction includes increased secretion of corticosterone and impairment of gonadotropins (17). The possible involvement of glucocorticoids in causing obesity has attracted much interest, following the observation that early adrenalectomy prevents obesity from developing, whereas glucocorticoid replacement can restore it (44). This dependence of obesity on glucocorticoids may relate to specific neuroendocrine abnormalities at hypothalamic level, rather than to the peripheral effects of steroid excess (see below).

Although some aspects of neuroendocrine function are similarly disturbed in the IDDM- and NIDDM-like models, they may arise in different ways and have different cause–effect relationships to diabetes. Any disorders in the IDDM-like syndromes must be secondary to direct or indirect effects of insulin deficiency, due to β-cell destruction. In NIDDM-like syndromes, the role of these disturbances is less clear. Many of these syndromes' major features (e.g., reduced energy expenditure, hyperphagia, obesity, and disordered secretion of insulin and pituitary hormones) could be explained by a primary abnormality in the hypothalamus. As discussed below, insulin may act on the hypothalamus to regulate various aspects of neuroendocrine function and energy metabolism, and it is possible that some of the hypothalamic disturbances in IDDM- and NIDDM-like syndromes could have a common basis in insulin deficiency at hypothalamic level. In the case of IDDM, these disturbances would be due to absolute deficiency of circulating insulin, whereas hypothalamic dysfunction in the NIDDM-like syndromes might be explained by insulin being unable to exert its normal actions on the hypothalamus because the relevant neurons are resistant to insulin action.

THE HYPOTHALAMUS AND DIABETES

The hypothalamus has an undisputed central role in the control of pituitary secretion, food intake, and, through its autonomic outputs to BAT and the pancreatic islets, in controlling energy expenditure and insulin secretion. It is therefore a logical place to begin the search for the origins of the neuroendocrine and energy balance disturbances in diabetes.

The hypothalamic pathways regulating pituitary secretion are relatively well defined, but those controlling nutritional state are still far from clear. Understanding was clouded for many years by misinterpretation of some classical studies, in which lesions of the ventromedial nucleus (VMH) and adjacent areas were found to cause hyperphagia and obesity, whereas damage to the lateral hypothalamic area (LHA) led to hypophagia and weight loss. These syndromes exist, but appear now to result from damage to neural pathways that pass through or close to these regions, rather than to destruction of specific satiety or feeding centers. Although the VMH and LHA are sensitive to various neurotransmitters that affect feeding behavior and body weight, other hypothalamic areas are undoubtedly important in regulating energy metabolism. The paraventricular nucleus (PVN) is now thought to be a key integrating center for a variety of metabolic and neural signals that relay information about nutritional state. Several neural pathways, containing various neurotransmitters, converge on the PVN, and many appetite-modulating neurotransmitters are at their most potent when injected into this nucleus. These substances include appetite-stimulating peptides such as NPY (125,126) and galanin (66) and the anorectic agent cholecystokinin (37), and nonpeptide neurotransmitters such as norepinephrine (73) and serotonin (119), which stimulate and inhibit feeding, respectively. The dorsomedial nucleus (DMH), suprachiasmatic nucleus (SCN), and medial preoptic area (MPO) are also implicated in the control of feeding behavior, energy expenditure, and body weight. The DMH may be important in integrating information from other hypothalamic regions including the VMH and LHA, whereas the SCN contains the pacemaker that drives the circadian rhythmicity that dominates virtually all metabolic and endocrine functions. The MPO may affect thermoregulation and food intake. Many interconnections are known to exist between these areas and other sites within and beyond the hypothalamus, although their precise functions are not yet clear. The complex subject of the functional neuroanatomy of the hypothalamus has recently been comprehensively reviewed (22).

Neurotransmitters in the Hypothalamus

The hypothalamus contains a veritable alphabet soup of peptide and nonpeptide neurotransmitters. These have many experimental actions that may hint at, but do not necessarily reveal,

their physiological roles in vivo. Functions as complex as feeding behavior and energy metabolism are undoubtedly controlled by many neurotransmitters interacting at different levels. There may or may not be a final common pathway, mediated by a single neurotransmitter, through which all other influences are channelled. In view of the rate at which new CNS peptides are being discovered, it is quite possible that the major players in these functions have not yet been identified.

The many neuropeptides that can affect energy balance have been extensively reviewed elsewhere (95). We do not propose to discuss this vast topic in any detail, but must draw attention to the fact that changes in numerous hypothalamic neurotransmitters have been reported in certain diabetic or obesity syndromes. These include CCK, CRF, somatostatin, and opioid peptides (148). Of particular interest are CRF and the opioid peptides. CRF has potent effects on energy metabolism in addition to its stimulation of corticotropin release, and CRF and NPY neurons are closely related in the hypothalamus (see below). Similarly, opioids and NPY may interact functionally in the control of energy balance (68), whereas hyperglycemia and/or insulin deficiency appear to affect specific classes of opioid receptors and/or the binding of the endogenous ligands at these receptors, and perhaps the synthesis of these neurotransmitters (50,76,79). Currently, it is not clear how these neurotransmitters relate to neuroendocrine function in diabetes, or to the altered activity of the NPY-containing pathways, which is the subject of the rest of this review. At present, NPY seems a convincing candidate for mediating the major neuroendocrine disturbances of diabetes, although its apparent importance may be eclipsed by other neurotransmitters in years to come.

NEUROPEPTIDE Y: AN INTRODUCTION

NPY exemplifies the incredible speed with which novel peptides can now be investigated. Since its discovery in 1982 (130), NPY has been sequenced and synthesized; its distribution has been extensively mapped throughout the CNS and elsewhere in several species; its three-dimensional structure and functional domains have been characterized in some detail; its gene and that of one of its receptors have been cloned and sequenced; and the first NPY receptor blockers have already been designed and produced. These advances have taken about one-sixth of the time that was needed for the same exercises to be completed for insulin. Many newly discovered peptides excite interest only transiently; NPY is one of those that continues to attract attention, as is evident from the 2000 papers published about it to date.

Like several other peptides, NPY was first identified by Tatemoto and Mutt (130) in an extract of porcine brain (a by-product of the Danish bacon industry), using a technique that identifies peptides that are amidated at the *C*-terminus. NPY contains 36 amino acid residues, including a tyrosine (=Y) residue at both ends and three other tyrosines in the body of the molecule (129). NPY belongs structurally to the family that includes pancreatic polypeptide (the patriarch) and peptide tyrosine-tyrosine (PYY), and in common with these, consists of a single peptide chain bent into a hairpin shape that brings both ends of the molecule together for receptor binding (46). NPY has been identified in many species including man, rat, and birds, and its structure is closely conserved across species. NPY is cleaved from a precursor (98 amino acids; M_r 11,032) derived from the gene (7.2 kb) that contains four exons (54,70). The portion of the gene upstream of the coding sequence contains various putative regulatory sites, including possible glucocorticoid-responsive elements (31).

NPY is found in many organs and tissues, notably the CNS, the sympathetic innervation to blood vessels throughout the body, and the gut and urogenital tract. In the brain, NPY is the most abundant neuropeptide yet identified and is found at particularly high concentrations in the hypothalamus, hippocampus, and cortex (5,32).

NPY in the Hypothalamus

Within the hypothalamus, NPY is concentrated in specific nuclei and regions. In situ hybridization shows that virtually the only hypothalamic neurons that synthesize NPY under normal circumstances are found in the arcuate nucleus (ARC) (96), which runs along the base of the third ventricle just above the median eminence (Fig. 1). The axons of most of these cells project upwards and rostrally, most passing through the LHA to end in the PVN; others terminate in the DMH and MPO. Tracing studies have shown that these latter areas, and especially the PVN, also receive NPY-containing fibers that arise from cell groups in the medulla (A_1, C_{1-3}) (115). The proportion of the NPY content of the PVN that is supplied by this extrinsic projection is not known. The medullary NPYergic neurons also synthesize catecholamines, whereas the intrahypothalamic ARC-PVN pathway does not contain catecholamines (7,115).

The NPY-containing pathways of the hypothalamus have the potential to interact with other neurotransmitters and humoral signals at various levels. The ARC, the site of NPY synthesis, is rich in releasing factors and other peptides and also contains plentiful insulin receptors (28). The latter are highly relevant to NPY's possible involvement in regulating energy balance (see below), although their precise anatomic and functional relationship to the NPY-containing cell bodies is not yet clear. Within the PVN, NPY-containing nerve terminals are clustered around cell bodies that synthesize corticotropin-releasing factor (CRF) (78), whose axons descend beside the third ventricle to end in the ARC. Elsewhere in the hypothalamus, NPY-containing pathways are closely associated with serotonergic fibers (e.g., in the PVN and SCN) (21,51,116) and, in the supraoptic nucleus, with magnocellular neurons that synthesize and release vasopressin (151).

NPY-binding sites have been demonstrated by autoradiography throughout the CNS (35,48); curiously, in contrast to the peptide's high concentration in the hypothalamus, the density of binding sites in this area seems relatively low. Pharmacologic studies have clearly distinguished two subtypes of NPY receptor

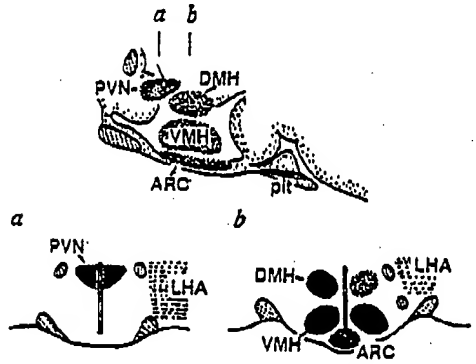

FIG. 1. Schematic longitudinal and frontal sections through the rat hypothalamus showing the principal areas involved in the control of energy balance. ARC, arcuate nucleus; DMH, dorsomedial nucleus; LHA, lateral hypothalamic area; PVN, paraventricular nucleus; VMH, ventromedial nucleus; plt, pituitary.

on the basis of their relative abilities to bind intact NPY and fragments of the molecule. Y_1 receptors, found in the CNS (including the hypothalamus) and postsynaptically at sympathetic nerve endings, preferentially bind intact NPY. Y_2 receptors occur throughout the brain and in specific peripheral tissues such as the spleen, and have a higher affinity for *C*-terminal fragments of NPY, including the specific agonist $NPY_{(13-36)}$ (34,134). The Y_1 receptor is of particular interest here because it (or a related variant) appears to mediate the intense hyperphagic action of NPY (41). The subtypes mediating NPY's other metabolic and neuroendocrine effects are not known. Signal transduction beyond the NPY Y_1 receptor is mediated by G-proteins (23) and, in common with others of this class, the receptor is a long, single-chain molecule that is thought to snake in and out of the cell membrane.

EXPERIMENTAL ACTIONS OF NPY

NPY has an impressive and diverse range of actions when injected into certain CNS regions, including the hypothalamus (Table 2). The potency and specificity of these effects suggest that some may reflect NPY's actions in vivo.

Effects of NPY on Energy Balance

Stimulation of feeding, perhaps the most spectacular effect of NPY, was first demonstrated by injection of the peptide into the third ventricle (24,75). More precise targeting studies have identified the PVN, and also the perifornical LHA, DMH, VMH and MPO, as sites most sensitive to NPY's hyperphagic action (121,122,125,126). When injected into the PVN, NPY and PYY are the most potent central appetite stimulants yet identified; on a molar basis, NPY is several hundred times more powerful than norepinephrine. Feeding can be stimulated three- to fourfold after a single injection, even in rats that are already satiated; indeed, the animals do not become refractory to the hyperphagic action of NPY when it is injected repeatedly, and obesity can be induced after several days of continued administration (123). As discussed below, obesity is attributable to reduced energy expenditure and possibly hyperinsulinemia, as well as to increased food intake. Rats stimulated to feed by central NPY injection show marked macronutrient preference, and selectively eat carbohydrate-rich food (122). During chronic NPY administration, fat intake is also relatively increased (120,123).

Many neuropeptides affect food intake and body weight experimentally (95), but NPY stands out against the generality of putative appetite-regulating factors because of the extreme potency of its hyperphagic effect, the behavioral specificity of this action (which simulates all aspects of normal hunger), and the accumulating evidence that the NPYergic ARC-PVN pathway is selectively stimulated under conditions when feeding normally increases. Further evidence that feeding is driven at least in part by NPY released in the hypothalamus has recently been provided by studies that sought to block the effects of endogenous NPY. Leibowitz et al. have reported that an NPY antagonist, PYX_2 [recently synthesized by Tatemoto's group (131)], injected into the PVN, significantly reduced food intake in freely fed rats and also when feeding was stimulated by concomitant injection of NPY (74). This response is evidently highly circumscribed, as we previously found PYX_2 to have no effect when injected into the third ventricle at high dosages of up to 1 μmol (McCarthy et al., unpublished observations). It has also been reported that a monoclonal antibody against NPY can significantly reduce feeding when injected into the third ventricle of fasted rats, presumably by immunoneutralizing NPY released in adjacent regions (68).

TABLE 2

CENTRAL EFFECTS OF NEUROPEPTIDE Y

Action	Sites
Stimulates feeding	PVN, VMH, DMH, LHA, fourth ventricle
Stimulated drinking	PVN
Reduces energy expenditure	PVN, MPO
Increases insulin secretion	PVN
Increases ACTH and corticosterone	PVN
Reduces growth hormone, thyrotropin and prolactin	third ventricle, ARC
Increases/reduces LH (depends on sex steroid levels)	third ventricle
Shifts circadian rhythms	SCN
Enhanced memory processing	third ventricle

NPY injected into the third ventricle, PVN, or MPO also reduces energy expenditure (13), an effect that may be explained by reduced firing of the sympathetic nerves that stimulate heat production in BAT (36). NPY injected into the PVN has a further effect relevant to obesity and diabetes, namely stimulation of insulin secretion (94). This is apparently mediated by increasing the activity of the vagal innervation that drives the β-cells, and would tend to promote triglyceride deposition in adipose tissue.

In view of its ability to increase energy intake, reduce energy expenditure, and stimulate insulin secretion, it is not surprising that repeated injection of NPY into the PVN results in obesity. These actions represent a concerted shift in the overall balance of the autonomic regulation of energy metabolism, in favor of the parasympathetic system. Such a shift was postulated some years ago by Jeanrenaud to lead to obesity in both genetically obese and VMH-lesioned rodents (58). Evidence that spontaneously increased activity of hypothalamic NPYergic pathways could lead to obesity is discussed below.

Other CNS Effects of NPY

NPY affects all anterior pituitary hormones when injected into selected hypothalamic regions or the third ventricle. It causes release of corticotropin and of corticosterone (135), an effect that apparently involves the release of CRF in the median eminence. This experimental action of NPY may be physiologically relevant, although its importance as a corticotropin secretagogue is not yet elucidated.

NPY has complicated effects on gonadotropin release that are greatly influenced by the prevailing sex steroid levels. NPY administered into the third ventricle of male rats inhibits LH release, an action that could be explained by the known interactions of NPY with neurons that modulate the secretion of gonadotropin-releasing hormone in the ARC; by contrast, it stimulates LH release when injected centrally into estrogen-primed female rats (61). Feedback regulation of NPY by sex steroids is suggested by the observation that castration reduces hypothalamic NPY concentrations (109) and NPY mRNA levels (133) and, more relevantly, decreases NPY secretion both in vitro (109) and in vivo (111). This complicated topic has been comprehensively reviewed by Kalra (62).

Other central endocrine effects of NPY injected into the third ventricle include inhibition of growth hormone, prolactin, and thyrotropin secretion (87). There is mild stimulation of glucagon coincident with insulin release when NPY is injected into the PVN (2). Administration of NPY into the supraoptic nucleus, in

which vasopressin-synthesizing neurons are surrounded by NPY-containing terminals, evokes vasopressin release (151).

NPY administered intrahypothalamically also stimulates active food-seeking behavior and drinking as well as feeding itself, and inhibits sexual behavior. Its numerous other CNS actions, beyond the scope of this review, include alterations in the level of arousal, respiration, pulse rate, and blood pressure (47), and an intriguing enhancement of memory when injected into the third ventricle (40).

NPY AND THE CONTROL OF ENERGY HOMEOSTASIS

As NPY appears to act within the hypothalamus in a concerted fashion to cause weight gain, it is tempting to speculate that one of its physiological roles might be to conserve body weight. If this was the case, the NPY-containing pathways involved might be expected to complete a homeostatic feedback loop, and therefore to be stimulated under conditions of negative energy balance or weight loss. This hypothesis has now been tested in several models, and appears to be supported by the available evidence.

In the wild, the commonest challenge to energy homeostasis must be starvation. Food-deprived rodents channel their activities into seeking food at the expense of sexual and other behaviors, overeat when food is found, and show adaptive reductions in BAT thermogenesis and overall energy expenditure. As discussed above, these changes are all elicited by NPY injected into the PVN and other hypothalamic sites, suggesting that the peptide could be involved. Food-deprived rats show marked increases in NPY concentrations in the ARC (12,110), with some studies reporting several-fold increases within 48 h of food withdrawal (12). NPY levels also rise in the PVN, DMH, and MPO. These rises in regional hypothalamic NPY levels are paralleled by increases in hypothalamic NPY mRNA (16,137), localized by in situ hybridization to the ARC (16); other brain regions (including the sparse neurons in the PVN and DMH that also express NPY) do not show any increases in NPY mRNA (85). These changes point to increased synthesis of NPY within the ARC and enhanced transport along the ARC-PVN/DMH projection to its sites of release. NPY release within the PVN of conscious, unrestrained rats is increased after 72 h of food deprivation, as has recently been demonstrated by push–pull sampling (60): NPY is secreted in large-amplitude pulses that contrast dramatically with the rather flat release profile in freely feeding rats (Fig 2). Increased release of NPY within the hypothalamus of food-deprived rats is further suggested indirectly by a 30% reduction in high-affinity NPY receptor numbers, consistent with downregulation (43). Increases in regional NPY concentrations and NPY release within the PVN fall towards normal after refeeding (60,110), although these changes are not immediately reversible; NPY receptor numbers, for example, remain downregulated for at least 48 h afterwards (43). At this time, the rats are still underweight; teleologically, it would be appropriate for a homeostatic circuit that defends body weight to remain overactive until any deficit in energy balance has been fully corrected. Chronic food restriction, like short-term fasting, also increases regional hypothalamic NPY concentrations and NPY mRNA levels (16).

Overall, these observations strongly suggest that an inadequate energy intake selectively stimulates the ARC-PVN projection. Similar NPY changes occur in other conditions that induce major energy deficits, namely exercise, lactation, and insulin-deficient diabetes. Rats trained to run intensively on an exercise wheel (enough to increase energy expenditure by ≈40%) showed significant increases in NPY concentrations in the ARC, DMH, and MPO, which were very similar to those found in a separate

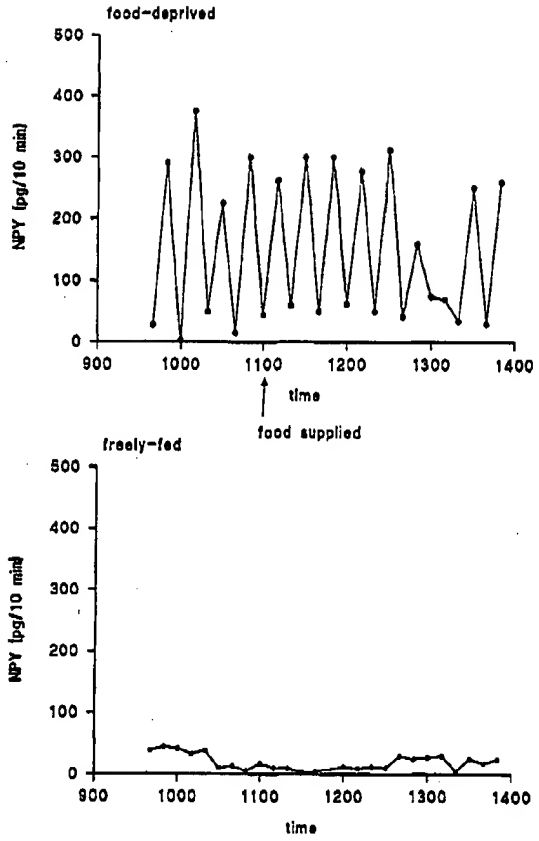

FIG. 2. NPY release within the PVN, measured in vivo by push–pull sampling. These representative profiles [redrawn from Kalra et al. (60)] show the marked increase in NPY secretion in rats deprived of food for 72 h. Food was presented to food-deprived rats at 1100 h.

nonexercised group that was underfed to match the weight loss of the runners (77). Lactating rats, which display striking hyperphagia (39) and reduced BAT thermogenesis (132) to compensate for the energy drain of milk production and mammary gland hypertrophy, have increased NPY concentrations in the ARC, PVN, VMH, and DMH, together with increased NPY mRNA levels in the ARC (83,102).

The common factor in the above conditions, and in insulin-deficient diabetes (below), is negative energy balance, whether induced by inadequate intake or excessive expenditure. All these conditions induce hyperphagia and reduce BAT thermogenesis, which could be viewed as homeostatic responses to counteract the energy deficits. It seems reasonable to suggest that these adaptive changes may be driven, at least in part, by increased activity of the NPYergic neurons in the ARC, with enhanced NPY synthesis, transport, and release in the NPY-sensitive PVN, DMH, and MPO. The ARC-PVN projection may therefore serve an important function in energy homeostasis.

These observations have focused on the intrahypothalamic ARC-PVN/DMH pathway. The importance of the medullo-PVN projection in controlling energy balance is uncertain. Selective lesioning of this projection does not affect spontaneous food intake, although the hyperphagic response to NPY injected into the PVN is enhanced (108); this hints at some regulatory function,

but NPY and NPY mRNA levels in this input have not yet been investigated.

HYPOTHALAMIC NPY IN ANIMAL DIABETES

We shall now review the evidence that changes in hypothalamic NPY could explain various neuroendocrine and behavioral changes in diabetic rodents. The insulin-deficient and hyperinsulinemia/obesity diabetic syndromes differ fundamentally, and will therefore be discussed separately. However, it will become apparent that the activity of the NPYergic projection arising in the ARC is increased in both types of syndrome. The different ways in which this projection could be stimulated in these contrasting syndromes are discussed below.

NPY in Insulin-Deficient Diabetes

Given its experimental actions, NPY is an obvious candidate for mediating the hypothalamic disturbances of insulin-deficient diabetes (Tables 1 and 2). The first evidence for this emerged from a rather speculative study of STZ-diabetic (STZ-D) rats (150) in which a dozen peptides were assayed in tissue blocks from the medial and lateral hypothalamus, rather than in specific nuclei. Of the peptides studied, only NPY showed consistent changes compared with nondiabetics: diabetic rats displayed significantly higher NPY concentrations in the central hypothalamus (which included the ARC, PVN, DMH, and MPO) and in the lateral hypothalamus (comprising mainly the LHA and the lateral preoptic area). These NPY increases became significant after 4 weeks of diabetes and were still present after 6 and 14 weeks. Parallel immunocytochemical studies showed increased NPY-immunoreactive staining of nerve fibers in the medial hypothalamus, and intense NPY staining in distended cell bodies in the supraoptic nucleus. In a subsequent study, significantly elevated NPY concentrations were found in the central hypothalamus of spontaneously diabetic BB/E Wistar rats whose insulin dosage had been deliberately reduced to allow hypoinsulinemia, hyperglycemia, and hyperphagia to develop (147). This demonstrates that increased hypothalamic NPY concentrations are a common feature of insulin-deficient diabetes, rather than a toxic effect of STZ.

We suggested initially that this change might reflect increased activity of NPY-containing pathways in the hypothalamus and that the peptide might mediate hyperphagia, polydipsia, and pituitary dysfunction in insulin-deficient diabetes. From the sequence of changes in hypothalamic NPY and blood glucose and insulin concentrations, we speculated that insulin deficiency, rather than hyperglycemia, might be the stimulus for NPY. We subsequently used a micropunch technique to localize the increased NPY concentrations to specific hypothalamic regions and found consistent elevations in the ARC at 3–14 weeks of STZ-diabetes, with additional increases in the PVN, DMH, MPO, VMH, and LHA (146). Northern blotting of hypothalamic blocks showed a fivefold increase in NPY mRNA in STZ-diabetic rats (105) and subsequently in diabetic BB/E Wistar rats (59), indicating that NPY gene expression (and presumably NPY synthesis) was increased in the hypothalamus.

Other groups have confirmed these observations. Sahu et al. found increased NPY concentrations in the ARC, PVN, VMH, DMH, and MPO of STZ-D and BB rats with long-standing (6 months' duration) diabetes (112). They also found that hypothalamic tissue from diabetic rats released more NPY in vitro in response to K^+-induced depolarization than controls (112). White et al. have demonstrated that hypothalamic NPY mRNA levels are raised (138) and Marks et al. confirmed that this increase in NPY gene expression is confined to the ARC (86). We have recently found that NPY receptor numbers in hypothalamic blocks are reduced in STZ-diabetic rats of 3 weeks' duration; as in food deprivation, we suggest that this indicates increased release of endogenous NPY within the hypothalamus (43). Push–pull sampling (113) and a modified microdialysis technique with improved NPY recovery (67) have recently demonstrated that release of NPY in the PVN is enhanced in diabetic rats. Stanley (124) and Wilding (142) have found that the feeding response to intrahypothalamic NPY is blunted in STZ-diabetic rats, which could also be explained by enhanced release of NPY in the hypothalamus; this could downregulate NPY receptors in the PVN and other sites, consistent with our own findings, and so reduce the sensitivity to exogenous NPY.

Overall, these observations point to increased activity of the ARC-PVN/DMH projection, with increased NPY synthesis, transport along this pathway, and release in the PVN. NPY could be plausibly involved in driving hyperphagia, polydipsia, reduced BAT thermogenesis, and in increased corticosterone secretion in insulin-deficient diabetes (Table 2); its role in abnormalities of growth hormone, gonadotropin, and other endocrine axes must remain speculative until its effects on these hormones have been localized more precisely within the hypothalamus. Also uncertain is the contribution of the extrinsic medullo-PVN input, and particularly the extent to which it could account for increases in NPY secretion in the PVN.

NPY in Hyperinsulinemic/Obese Diabetes Syndromes

The first studies were conducted in *ob/ob* mice, using blocks of hypothalamic tissue. Hypothalamic neurotensin concentrations were consistently significantly lower in the obese mutants than in lean controls, but NPY levels did not differ (145). This negative finding cannot exclude significant NPY increases confined to discrete nuclei, especially as a subsequent Northern blotting study found raised NPY mRNA levels (together with reduced neurotensin mRNA) in *ob/ob* mice (141). Further detailed studies of regional hypothalamic NPY levels and receptor characteristics are clearly required to resolve this uncertainty.

In rats, the fatty (*fa/fa*) Wistar is the closest approximation to human NIDDM in which hypothalamic NPY has been reported. NPY levels were increased in the PVN of these animals (1), which displayed the typical syndrome of moderately severe hyperglycemia (22 mmol/l), plasma insulin levels raised to 10 times normal, and obesity. NPY mRNA, receptors, and release have not yet been investigated in this model.

Certain genetically obese rats, which share some common features with the NIDDM models, also show evidence of increased ARC-PVN/DMH pathway activity. Fatty (*fa/fa*) Zucker rats have increased NPY concentrations in regions along the pathway (ARC, PVN, DMH, VMH) and also in the SCN (11,91). [Initial studies in fatty Zucker rats (144) had not revealed any NPY increases in hypothalamic blocks, which highlights the importance of focusing on individual nuclei when studying the hypothalamus.] Fatty Zucker rats also have increased NPY mRNA concentrations in the ARC (114), downregulated NPY receptor numbers in hypothalamic tissue (89), and attenuated feeding responses to NPY injected intracerebroventricularly (89). These findings closely parallel those in STZ-D rats and point firmly to increased NPY synthesis, transport, and release in the hypothalamus. Corpulent (*cp/cp*) JCR:LA rats also have higher NPY levels than in lean controls in the ARC, but not in any other regions (149); further investigations of hypothalamic NPY in this strain are awaited.

It must be emphasized once again that *fa/fa* and *cp/cp* rats have only mild glucose intolerance and are not truly diabetic.

Nonetheless, they overlap with the NIDDM models in that they display hyperphagia, reduced energy expenditure, hyperinsulinemia, and insulin resistance. As discussed below, the insulin resistance common to these syndromes may be crucially important in activating the ARC-PVN/DMH projection.

WHAT REGULATES HYPOTHALAMIC NPY IN DIABETES?

It is important to identify the factors that stimulate the NPYergic ARC-PVN/DMH pathway in the various models of diabetes. This exercise should not only clarify how this important neurotransmitter is regulated, but may also throw much-needed light on the general control of energy metabolism, pituitary secretion, and other hypothalamic functions in health and disease.

As diabetes leads to changes in many metabolites and hormones as well as in energy balance, electrolyte status, and so on, it is difficult to single out the prime mover(s) that might stimulate NPY neurons in the ARC. Possible candidates include:

1. Changes in circulating insulin concentrations. Any theory must explain how diametrically opposite changes in insulinopenic and hyperinsulinemic syndromes both stimulate the ARC-PVN/DMH projection.
2. Changes in energy substrates or metabolites, of which hyperglycemia is the most obvious.
3. Changes in glucocorticoids, thyroid, or other hormones.
4. Other factors, including stress, body weight changes (which also contrast markedly between the various syndromes), state of hydration, and so on.

Currently, the most attractive hypothesis appears to be that the ARC-PVN/DMH projection is normally inhibited by insulin, and that its overactivity in diabetic rats may be explained by failure of insulin to exert this action. However, not all the available evidence sits comfortably with this proposal, and other possibilities must still be entertained. The evidence for and against insulin, glucose availability, and glucocorticoids will now be reviewed; the other factors listed above have not yet been investigated.

Insulin, the CNS, and NPY

Intuitively, insulin should have no place in the brain, which has long been regarded as an insulin-independent tissue. In recent years, however, it has become clear that insulin is found in specific brain regions, mostly in association with insulin receptors. Particularly high concentrations of insulin receptors are found in the olfactory bulb and hypothalamus, notably in the median eminence-ARC region (28). CNS insulin receptors are now known to comprise two subtypes; one is identical to that in peripheral tissues, and is localized to glial cells (3), whereas neurons express a structurally distinct, lower molecular weight variant that is unique to the CNS (53).

Much of the insulin in the brain probably enters from the circulation, especially through specialized regions in which the blood–brain barrier is incomplete. These areas include the circumventricular organs lying around the third ventricle, notably the median eminence; in vivo autoradiographic studies have demonstrated convincingly that radiolabeled insulin injected intravenously can penetrate both the median eminence and ARC (9). There is some evidence that certain neurons can synthesize insulin in vitro (25), and some investigators have demonstrated insulin mRNA—albeit at very low levels—in specific brain regions, particularly the periventricular region (154). Although the significance of any insulin produced by the brain has not been systematically investigated, it is generally assumed that it is overshadowed by insulin derived from the bloodstream.

The possible functions of insulin in the CNS are even more controversial than its origins. Porte, Baskin, Woods, and colleagues have assembled a convincing case that insulin acts centrally to regulate energy balance and body weight. Insulin injected into the third ventricle of various species reduces food intake and ultimately body weight (18,152), whereas injection of insulin antiserum into the VMH can induce hyperphagia (127). Central injections of insulin can also stimulate the sympathetic innervation supplying BAT (107), and so increase energy expenditure. As overall circulating levels of insulin generally parallel body fat content, and as it can apparently gain access to the hypothalamic regions that control energy homeostasis (9), insulin would seem well qualified to convey information about the body's energy stores to the CNS. Furthermore, as its central actions would tend to reduce body weight and fat stores, it could act homeostatically via the hypothalamus to limit excessive weight gain.

Insulin could affect hypothalamic cells directly, as certain neuron populations (e.g., in the LHA) alter their firing responses according to changes in ambient insulin concentrations in vitro (100). Alternatively, insulin could influence neuronal activity by modulating the availability of glucose or other energy substrates in the CNS. The conventional view that the brain does not require insulin has been challenged by the demonstration that hyperinsulinemia stimulates glucose uptake into specific regions, including the DMH, VMH, and anterior hypothalamic area (AHA) (81), some of which contain glucose-responsive neurons. Taken together, these observations hint strongly at a role for insulin in the central regulation of metabolism and energy balance, although its mechanisms of action are yet to be defined. NPYergic neurons in the hypothalamus may be some of the pieces missing from this puzzle.

Insulin–NPY interactions. Insulin and NPY have mutually antagonist central effects on energy homeostasis, and several studies, which have manipulated insulin levels in the circulation or locally in the hypothalamus, have suggested that insulin may inhibit the ARC-PVN/DMH projection. Circumstantial evidence includes the observation that hypoinsulinemia is a common feature in all the conditions of energy deficit—starvation, diabetes, intense exercise, and lactation—in which the projection appears to be stimulated. It has been proposed that insulin acts directly on NPY-synthesizing neurons in the ARC, because Schwartz and colleagues have reported that insulin injected into the third ventricle prevented the increase in NPY mRNA levels that normally occurs in food-deprived rats (118). Intriguingly, insulin exerted this inhibitory effect in normal Wistar and in lean (Fa/Fa) Zucker rats, but not in fatty (*fa/fa*) Zucker rats (117); the implications of this apparent failure of insulin action in the obese mutants are discussed in detail below. Insulin can apparently enter the ARC from the circulation and so could, in theory, directly affect NPY-synthesizing neurons in this nucleus, although it is not known whether the insulin receptors in the ARC are carried by the NPY neurons; indeed, a substantial proportion of these insulin receptors are apparently found on catecholamine-containing fibers (140) that are distinct from the ARC-PVN/DMH projection (7).

The significance of Schwartz's observations (117,118) may be tempered by the fact that insulin injected into the third ventricle would reach the ARC neurons by passing through the ependyma lining the ventricular system, rather than having to follow the physiological route across the blood–brain barrier from the circulation, and might reach local concentrations far above those occurring in real life. To try to avoid these problems, we have examined the effects of physiologically relevant changes in cir-

culating insulin levels. In the first study, we determined how carefully monitored systemic insulin treatment affected regional hypothalamic NPY concentrations in rats fasted for 3 days. Relatively low insulin dosages (5 U/kg/day), titrated to avoid hypoglycemia, maintained circulating insulin concentrations in fasted rats at levels similar to those in freely fed controls (84). We found that the rise in NPY concentrations in the ARC normally induced by food deprivation was significantly attenuated in the insulin-treated, fasted group. This could be explained by circulating insulin entering the hypothalamus and inhibiting NPY synthesis in ARC neurons. On the other hand, higher insulin levels would undoubtedly have other peripheral metabolic effects, such as restraining the lipolysis and ketogenesis of starvation; therefore, we cannot exclude the possibility that other metabolites could mediate insulin's effects on hypothalamic NPY.

Two further studies have employed the euglycemic, hyperinsulinelc clamp technique, in which circulating insulin levels are raised by insulin infusion whereas glucose is given intravenously at a rate adjusted to maintain blood glucose concentrations within the normal range. Both studies indicate that, if insulin does affect the activity of the ARC-PVN/DMH projections, then its actions may be more subtle than simple inhibition of NPY synthesis. We initially examined the effects of 2.5 h of hyperinsulinemia on hypothalamic NPY levels in rats fasted for 24 h; this short-term exposure aimed to simulate the acute rise in insulin levels that would be anticipated on refeeding. Previous studies would suggest that hyperinsulinemia should, if anything, tend to reduce the activity of the ARC-PVN/DMH pathway, yet the hyperinsulinemic group showed a significant increase in NPY concentrations in the ARC, with no other changes elsewhere (82). The second experiment investigated NPY mRNA and regional hypothalamic NPY levels after 5 days of hyperinsulinemia in freely feeding, unrestrained rats infused with glucose through implanted cannulae. As with acute hyperinsulinemia, there was no fall in NPY levels in the ARC or in NPY mRNA levels; indeed, the only significant change from saline-infused normoinsulinemic controls was a slight increase in NPY concentrations in the PVN (30). These apparently paradoxical results are not necessarily inconsistent with the view that insulin reduces the activity of the ARC-PVN/DMH pathway. Levels in the ARC or PVN could increase, for example, if insulin inhibited the transport of NPY out of the ARC to its sites of projection, or blocked its release from endings in the PVN, so causing the peptide to accumulate in these regions. It must be conceded that the euglycemic, hyperinsulinemic clamp technique does not simply impose changes in circulating insulin concentrations, as it will also increase glucose uptake and utilization in tissues which are insulin dependent. As the latter include various brain regions that could communicate with and influence the ARC NPY-neurons, such indirect metabolic effects could obscure a direct inhibitory action of insulin per se.

Finally, we administered sc insulin at high doses (60 U/kg/day), which caused profound hypoglycemia. In common with previous reports, this caused intense hyperphagia, and repeated insulin injections for 7 days induced excessive weight gain (29). We found no changes in NPY concentrations in the ARC, PVN, or any other hypothalamic regions, in rats with either acute or chronic (7 days) hypoglycemia. This negative finding is in striking contrast to all the other hyperphagic conditions mentioned above and suggests that, whatever the mechanism that stimulates feeding in hypoglycemia, NPY may not be involved. We have proposed that hyperinsulinemia may have prevented activation of NPYergic pathways, and that this indirectly supports the view that hypoinsulinemia is a specific stimulus to hypothalamic NPY.

As the rats had free access to food throughout these studies, it could be argued that overeating could itself have reversed or obscured any alterations in hypothalamic NPY; this seems unlikely, however, as diabetic rats that are hyperphagic to a similar degree show markedly raised NPY levels (92).

Insulin and hypothalamic NPY in diabetes. In IDDM, the ARC-PVN/DMH projection could hypothetically be activated by the fall in circulating insulin levels and the resulting loss of insulin's postulated inhibitory effect. This seems entirely reasonable from the above evidence, but is difficult to prove. Not surprisingly, insulin replacement tends to normalize the increases in NPY mRNA and NPY levels and the downregulation of NPY receptors in STZ-diabetic rats (43,86,112).

These studies obviously cannot determine whether the ARC-PVN/DMH projection is stimulated directly by insulin deficiency itself, or indirectly by hyperglycemia or other sequelae. Hyperglycemia appears unlikely from studies of food restriction in STZ-diabetic rats, which was found to lower and even normalize glycemia without raising plasma insulin concentrations (92). In contrast to insulin treatment, food restriction of diabetic rats did not lower regional hypothalamic NPY levels to normal; indeed, these rose even further in the ARC, PVN, VMH, and LPO (92) (Fig. 3). This indicates that hyperglycemia is not the main stimulus to hypothalamic NPY; in fact, in view of the additional rise in rats rendered normoglycemic without any change in insulinemia, it is even possible that hyperglycemia exerts an inhibitory effect. These observations are consistent with the suggestion that low circulating insulin concentrations are responsible, and also rules out the possibility that increased hypothalamic NPY levels are merely the result of hyperphagia. However, the additional increases in regional NPY in food-restricted diabetics, whose circulating insulin levels were no lower than in freely feeding diabetics, argues that other factors operate in addition to, or perhaps rather than, hypoinsulinemia. The direct effects of insulin on hypothalamic NPY in diabetic rats will undoubtedly be tested by injecting the hormone intracerebroventricularly, but these studies will be subject to the same caveats and uncertainties as outlined above.

In NIDDM/obesity models, the inconsistency between the characteristic hyperinsulinemia and the overactivity of the ARC-PVN/DMH projection could be reconciled if the hypothalamus

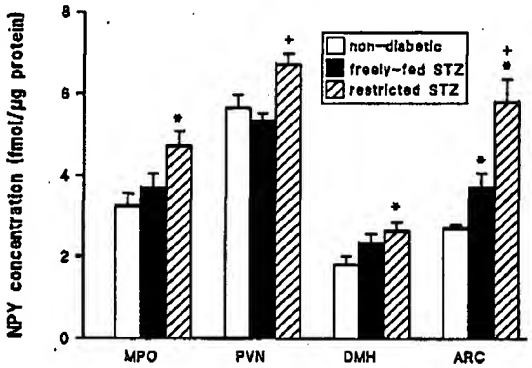

FIG. 3. Effects of food restriction on NPY concentrations in the ARC, DMH, PVN, and MPO of STZ-diabetic rats. Food restriction normalized glycemia, without affecting the low plasma insulin levels typical of STZ diabetes. NPY levels in these nuclei increased in freely fed STZ-D rats and rose further in those that were food restricted. Statistical significance of differences: $*p < 0.01$ vs. nondiabetics; $+p < 0.01$ vs. freely fed STZ-D group. [Redrawn from McKibbin et al. (92).]

(like certain peripheral tissues) was insensitive to insulin action. There is some experimental support for this notion in fatty Zucker rats, but not yet in other models closer to human NIDDM. Insulin injected intracerebroventricularly inhibits feeding in normal rats, including lean Zuckers, but not in fatty Zuckers (57). As mentioned above, intracerebroventricular insulin inhibited fasting-induced rises in NPY mRNA in the ARC of normal rats, but not fatty Zuckers (117). The ARC NPY neurons could therefore be resistant to inhibition by insulin. It was suggested by Porte and colleagues that hypothalamic insulin resistance could be due to defective insulin receptors, as insulin binding was reduced in the hypothalamus (and olfactory bulb) of fatty Zucker rats, compared with lean Zuckers (38). However, the situation is not yet clear-cut, as other studies have reported normal insulin receptor numbers in the whole hypothalamus and increased receptors in the ARC of fatty Zucker rats (52,139). Given the uncertainties regarding insulin's function in the CNS, there are few robust methods to assess changes in insulin action in obesity. In common with insulin receptors in other tissues, the brain insulin receptor has intrinsic tyrosine kinase activity, and can phosphorylate exogenous protein substrates at tyrosine residues. The distribution of phosphotyrosine-containing proteins in the brain is closely related to the distribution of insulin receptors, and has been shown to rise in certain brain regions following hyperinsulinemia. Interestingly, this rise was reduced in the hippocampus and olfactory bulb of fatty Zuckers compared to lean Zuckers, although the hypothalamus was apparently normal in the obese mutants (97,98).

Although glucose uptake in the brain is largely independent of the action of insulin, insulin-enhanced glucose utilization has been demonstrated in discrete brain regions (33) using the 2-deoxyglucose (2-DG) uptake technique, combined with image analysis. Hyperinsulinemic fatty Zucker rats show reduced 2-DG uptake throughout most gray matter areas compared to lean rats. These differences were most marked in the hypothalamus and autonomic pathways, including the nuclei shown to be responsive to insulin in normal rats (33). The exact pathophysiological significance of these findings is unclear, but may reflect, at least in part, abnormal action of insulin in the hypothalamus. Overall, these experimental results give some support to the hypothesis that insulin action in the hypothalamus is abnormal in obesity and NIDDM. There is some evidence that the ARC-PVN/DMH projection could be activated in these states by failure of insulin to exert its inhibitory effect, but the case is by no means proven.

Glucose and other metabolites. Glucose is a major metabolic fuel and its circulating levels may be an endogenous cue for feeding, as a small but significant decline has been found to occur before the onset of spontaneous eating (20). Ambient glucose concentrations can affect the activity of certain hypothalamic neurons directly (93), although the effects of glucose on NPY neurons in the ARC or elsewhere have not yet been investigated. As discussed above, glucose entry to specific brain regions may be enhanced by insulin, indicating that these two potential metabolic signals could interact in the CNS. Theoretically, NPY neurons could be stimulated by reduced glucose availability, as would occur during prolonged starvation or perhaps during the transient glycemic fall postulated to initiate feeding (20). There is some evidence for this, in that neuroglycopenia induce by 2-DG (used here in high doses as an inhibitor of glucose utilization, rather than a tracer for tissue uptake of glucose) has been reported by Leibowitz's group to increase NPY concentrations in the ARC (4). This could reflect increased NPY synthesis in the ARC neurons, and increased activity of the ARC-PVN/DMH projection has accordingly been implicated in the intense hyperphagia induced by neuroglycopenia. By contrast, we did not find 2-DG administration, at doses which stimulated feeding, to increase NPY concentrations in the ARC-PVN/DMH projection, although levels in the VMH were slightly but significantly increased (88). The reason for this inconsistency is not clear; further studies of NPY mRNA levels and NPY release in the PVN and other NPY-sensitive sites may provide an answer. As mentioned above, neuroglycopenia due to insulin-induced hypoglycemia did not affect NPY levels in the ARC or elsewhere (29), but the outcome may have been influenced by the potentially inhibitory effect of hyperinsulinemia.

In diabetes, glucose entry to insulin-sensitive neurons could be impaired, either through absolute insulin deficiency in IDDM, or through tissue insulin resistance in the NIDDM models, as discussed above. There is some evidence that the glucostat regions of the hypothalamus are insulin-dependent (136), although it is now apparent that glucose sensors exist not only in other brain regions, but also in extra-CNS sites such as the liver (45). In theory, intracellular glucose levels in these sites could be reduced in diabetes, leading to the starvation in the midst of plenty invoked for peripheral tissues. The study of food deprivation in STZ-diabetic rats cited above (92) excludes the possibility that hyperglycemia is a stimulus to hypothalamic NPY. In fact, as NPY concentrations in the ARC-PVN/DMH projection rose even higher following food deprivation (which normalized glycemia without increasing insulin levels), gross hyperglycemia may have partially inhibited these cells; glucose could have entered them to some extent through a mass action effect, even though insulin levels remained low.

Glucose naturally attracts most attention in the context of diabetes, but the possible importance of other metabolites must not be neglected. In particular, the products of lipolysis (free fatty acids (FFA) and glycerol) and the ketone bodies produced by FFA oxidation could plausibly be involved. Circulating levels of these metabolites are raised in fasting and insulin-dependent diabetes, and FFA concentrations are also increased in fatty Zucker rats (42). We are currently investigating the possible role of these substances in stimulating the ARC-PVN/DMH projection in diabetes by using inhibitors of lipolysis and ketogenesis.

Role of Glucocorticoids

As previously described, many anatomic and functional interactions have been identified between NPY and the hypothalamo–pituitary–adrenocortical axis, notably with CRFergic neurons in the PVN and ARC (78). Like many other neurons, NPY-containing cells in the ARC carry glucocorticoid receptors (55), and there is some evidence that glucocorticoids may stimulate, whereas adrenalectomy inhibits, NPY synthesis in the ARC (31,54). Dexamethasone treatment of normal rats has been reported to increase NPY concentrations in the mediobasal hypothalamus, which includes the ARC (27). On the other hand, we did not find either acute or chronic dexamethasone treatment to increase NPY levels in the ARC, although concentrations were significantly increased in the LHA and PVN, respectively (90). These studies can all be criticized because they all employed pharmacological doses of this powerful synthetic glucocorticoid, which, as well as simulating endogenous glucocorticoids, also induces insulin resistance, catabolism, and weight loss. All these secondary changes are known to affect the ARC-PVN/DMH pathway, which makes it particularly difficult to dissect out any effect of glucocorticoids per se; careful systematic studies employing physiological levels of endogenous glucocorticoids (corticosterone in rodents) have not yet been reported.

Glucocorticoids and NPY in IDDM. Glucocorticoids could be involved in stimulating the ARC-PVN/DMH projection in

IDDM, in which several studies have found corticosterone levels to be elevated (101). However, the relationship between raised glucocorticoids, hypothalamic NPY, and energy balance in IDDM models is puzzling. Increased corticosterone secretion would presumably be driven ultimately by increased CRF release within the hypothalamus, which might be predicted to cause hypophagia, increased BAT thermogenesis, and perhaps reduced NPY synthesis in the ARC (6,10). The fact that these are the opposite of the changes that occur in diabetes emphasizes the gaps that still remain in our knowledge of hypothalamic regulation, although it is possible that anatomically close, but functionally separate, CRFergic pathways mediate these various responses.

We have attempted to unravel the effect of glucocorticoids on the ARC-PVN/DMH projection in STZ-diabetes using mifepristone (RU 486), a powerful glucocorticoid receptor blocker, which also has powerful antiprogestagen activity. Mifepristone did not reduce NPY levels in the ARC or any other hypothalamic nucleus of diabetic rats, and neither was there any diminution of hyperphagia (19). Although this argues against involvement of glucocorticoids, the study is incomplete without measurements of NPY mRNA and of NPY release in the PVN. Furthermore, the outcome may have been confounded by the antiprogestagen action of mifepristone, even though we studied male rats to minimize this potential interference.

Glucocorticoids and NPY in obesity/NIDDM. The obesity syndromes (*fa/fa* rats and *ob/ob* mice) are more convincingly glucocorticoid dependent to some extent, in that adrenalectomy will prevent excessive weight gain, an effect reversible with glucocorticoid treatment (44). Plasma corticosterone levels are raised in the obese mutants but, as in STZ-diabetes, the events leading to glucocorticoid secretion are uncertain, especially as the production and/or release of CRF appears to be impaired in the *fa/fa* rat (99). Mifepristone reduces hyperphagia and obesity in *fa/fa* Zucker rats (69), yet appears to have little effect on the increased NPY mRNA levels in the obese mutants (104). A recent study found that obesity developed in transgenic mice in which the glucocorticoid receptor had been "knocked out" (103): as yet, the possible contribution of changes in hypothalamic NPY to this intriguing model has not been investigated.

Overall, although glucocorticoids have the potential to affect NPY-synthesizing neurons in the ARC, there is not yet a convincing body of evidence that they stimulate the ARC-PVN/DMH projection in either IDDM or obesity/NIDDM.

CONCLUSIONS AND RELEVANCE TO HUMAN DIABETES

How important are the NPYergic cells of the ARC-PVN/DMH projection in mediating the hypothalamic disturbances of diabetes? There is still uncertainty about the place of these relatively few cells, and of the peptide itself, in the hierarchy of transmitters that control hypothalamic function. These important questions could be resolved by selectively "knocking out" the projection using various techniques that have recently been developed. Use of NPY receptor blockers is at present hampered by uncertainty about the receptor subtype to be targeted, and by the low potency and poor selectivity of the antagonists currently available. Immunoneutralization is an alternative, but is also nonphysiologic. Perhaps the most attractive option is the use of genetic manipulation, for example, using antisense oligonucleotides injected intracerebroventricularly. This approach has proved spectacularly successful in inhibiting the expression of the NPY Y_1 receptor in the rat cortex, but selective targeting of the NPY-synthesizing cells of the ARC-PVN/DMH projection (while not affecting the other NPYergic neurons in the brain) will undoubtedly be problematic.

Finally, will all this work be consigned to the category of interesting phenomenonology, or can we extract something of use to treat human diabetes? In human IDDM, the clinically important problems attributable to hypothalamic dysfunction include reduced fertility and excessive growth hormone secretion, which has been implicated in the problems of poor diabetic control overnight and during puberty. The former can be largely alleviated by tightening glycemic control, and various therapeutic approaches (e.g., anticholinergic drugs such as pirenzepine) have been used to treat the latter; as yet, the possible involvement of NPY in these abnormalities has not been investigated. It is probably in NIDDM that deeper knowledge of the intricacies of hypothalamic control will be best rewarded, perhaps by the development of novel antiobesity drugs. Should NPY prove to be pivotal in the regulation of energy homeostasis in rodents, and should it be similarly important in man, then NPY antagonists would be rational and potentially highly effective antiobesity drugs, as they should both inhibit appetite and increase energy expenditure. As emphasized by the recent interim results of the UK Prospective Diabetes Study, routine dietary management of NIDDM succeeds in fewer than 20% of newly diagnosed patients (Turner, communication to British Diabetic Association, September 1993). When successful, dietary measures can reduce glycemia, blood pressure, and lipid levels as well as body weight, and these favorable changes are reflected in increased life expectancy (71). An effective and safe antiobesity drug would therefore revolutionize the management of NIDDM.

There are several conceptual and practical leaps in logic between our current state of knowledge and the possible clinical use of NPY antagonists. These include the lack so far of any systematic studies of NPY's possible functions in the human hypothalamus; the need to develop specific antagonists to the (unknown) NPY receptor subtype that mediates NPY's metabolic effects; and the pharmacokinetic challenges of ensuring that such drugs can be administered simply, absorbed reliably, and act selectively on a relatively tiny target in a key area of the brain. Nevertheless, there have been recent notable successes in refining analogues of peptides such as somatostatin and insulin, and optimizing their pharmacological properties. These encouraging precedents suggest that our rapidly expanding understanding of NPY may soon be exploited in the treatment of human obesity and NIDDM.

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FILE 'MEDLINE' ENTERED AT 16:25:44 ON 30 JUL 2001

FILE LAST UPDATED: 23 JUL 2001 (20010723/UP). FILE COVERS 1958 TO DATE.

On April 22, 2001, MEDLINE was reloaded. See HELP RLOAD for details.

MEDLINE now contains new records from the former NLM HEALTH STAR
database. These records have an Entry Date and Update Date of 20010223.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
MeSH 2001 vocabulary. Enter HELP THESAURUS for details.

The OLDMEDLINE file segment now contains data from 1958 through 1965.
Enter HELP CONTENT for details.

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Basic Index. See HELP SFIELDS for details.

THIS FILE CONTAINS CAS REGISTRY NUMBERS FOR EASY AND ACCURATE
SUBSTANCE IDENTIFICATION.

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=> s beacon
        90 BEACON
        67 BEACONS
L1      137 BEACON
           (BEACON OR BEACONS)

=> s l1 and gene or peptide
     506000 GENE
     325074 GENES
     614561 GENE
           (GENE OR GENES)
     217356 PEPTIDE
     137058 PEPTIDES
     291340 PEPTIDE
           (PEPTIDE OR PEPTIDES)
L2   291367 L1 AND GENE OR PEPTIDE

=> s l1 and l2
L3       30 L1 AND L2

=> duplicate remove
ENTER L# LIST OR (END):l3
PROCESSING COMPLETED FOR L3
L4       30 DUPLICATE REMOVE L3 (0 DUPLICATES REMOVED)

=> d 1- ibib, abs
YOU HAVE REQUESTED DATA FROM 30 ANSWERS - CONTINUE? Y/(N):y

L4  ANSWER 1 OF 30    MEDLINE
ACCESSION NUMBER:  2001209653    MEDLINE
DOCUMENT NUMBER:   21172531   PubMed ID: 11274026
TITLE:          Semiautomated DNA mutation analysis using a robotic
                workstation and molecular beacons.
AUTHOR:         Smit M L; Giesendorf B A; Vet J A; Trijbels F J; Blom H J
CORPORATE SOURCE:  University Medical Centre St. Radboud, 424 Department of
                Paediatrics, 6500 HB Nijmegen, The Netherlands.
SOURCE:         CLINICAL CHEMISTRY, (2001 Apr) 47 (4) 739-44.
                Journal code: DBZ; 9421549. ISSN: 0009-9147.
PUB. COUNTRY:      United States
                Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:       English
FILE SEGMENT:      Priority Journals
ENTRY MONTH:       200104
ENTRY DATE:        Entered STN: 20010425
                Last Updated on STN: 20010425
                Entered Medline: 20010419
AB  BACKGROUND: Our increasing knowledge of the genetic basis of inheritable
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diseases requires the development of automated reliable methods for high-throughput analyses. METHODS: We investigated the combination of semiautomated DNA extraction from blood using a robotic workstation, followed by automated mutation detection using highly specific fluorescent DNA probes, so-called molecular beacons, which can discriminate between alleles with as little as one single-base mutation. We designed two molecular beacons, one recognizing the wild-type allele and the other the mutant allele, to determine genotypes in a single reaction. To evaluate this procedure, we examined the C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene, which is associated with an increased risk for cardiovascular disease and neural tube defects. DNA was isolated from 10 microL of fresh EDTA-blood samples by use of a robotic workstation. The DNA samples were analyzed using molecular beacons as well as conventional methods. RESULTS: Both methods were compared, and no differences were found between outcomes of genotyping. CONCLUSIONS: The described assay enables robust and automated extraction of DNA and analysis of up to 96 samples (10 microL of blood per sample) within 5 h. This is superior to conventional methods and makes it suitable for high-throughput analyses.

L4 ANSWER 2 OF 30 MEDLINE
ACCESSION NUMBER: 2001092648 MEDLINE
DOCUMENT NUMBER: 20578194 PubMed ID: 11134272
TITLE: Establishment of new transmissible and drug-sensitive human immunodeficiency virus type 1 wild types due to transmission of nucleoside analogue-resistant virus.
AUTHOR: de Ronde A; van Dooren M; van Der Hoek L; Bouwhuis D; de Rooij E; van Gemen B; de Boer R; Goudsmit J
CORPORATE SOURCE: Department of Human Retrovirology, Academic Medical Center, University of Amsterdam, 1105 AZ Amsterdam, The Netherlands.. ronde@amc.uva.nl
SOURCE: JOURNAL OF VIROLOGY, (2001 Jan) 75 (2) 595-602. Journal code: KCV. ISSN: 0022-538X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200101
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010125
AB Sequence analysis of human immunodeficiency virus type 1 (HIV-1) from 74 persons with acute infections identified eight strains with mutations in the reverse transcriptase (RT) gene at positions 41, 67, 68, 70, 215, and 219 associated with resistance to the nucleoside analogue zidovudine (AZT). Follow-up of the fate of these resistant HIV-1 strains in four newly infected individuals revealed that they were readily replaced by sensitive strains. The RT of the resistant viruses changed at amino acid 215 from tyrosine (Y) to aspartic acid (D) or serine (S), with asparagine (N) as a transient intermediate, indicating the establishment of new wild types. When we introduced these mutations and the original threonine (T)-containing wild type into infectious molecular clones and assessed their competitive advantage in vitro, the order of fitness was in accord with the in vivo observations: 215Y < 215D = 215S = 215T. As detected by real-time nucleic acid sequence-based amplification with two molecular beacons, the addition of AZT or stavudine (d4T) to the viral cultures favored the 215Y mutant in a dose-dependent manner. Our results illustrate that infection with nucleoside analogue-resistant HIV leads in newly infected individuals to mutants that are sensitive to nucleoside analogues, but only a single mutation removed from drug-resistant HIV. Such mutants were shown to be transmissible, stable, and prone to rapid selection for resistance to AZT or d4T as soon as antiretroviral therapy was administered. Monitoring of patients for the presence of new HIV-1 wild types with D, S, or N residues at position 215 may be warranted in order to estimate the threat to long-term efficacy of regimens including nucleoside analogues.

L4 ANSWER 3 OF 30 MEDLINE
ACCESSION NUMBER: 2001225945 MEDLINE
DOCUMENT NUMBER: 21091152 PubMed ID: 11161323

TITLE: Use of real-time polymerase chain reaction and molecular beacons for the detection of Escherichia coli O157:H7.
AUTHOR: Fortin N Y; Mulchandani A; Chen W
CORPORATE SOURCE: Department of Chemical and Environmental Engineering, University of California, Riverside, CA 92521, USA.
SOURCE: ANALYTICAL BIOCHEMISTRY, (2001 Feb 15) 289 (2) 281-8. Journal code: 4NK; 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200104
ENTRY DATE: Entered STN: 20010502
Last Updated on STN: 20010502
Entered Medline: 20010426

AB Molecular beacons (MBs) are oligonucleotide probes that fluoresce upon hybridization. In this paper, we described the development of a real-time PCR assay to detect the presence of Escherichia coli O157:H7 using these fluorogenic reporter molecules. MBs were designed to recognize a 26-bp region of the rfbE gene, coding for an enzyme necessary for O-antigen biosynthesis. The specificity of the MB-based PCR assay was evaluated using various enterohemorrhagic (EHEC) and Shiga-like toxin-producing (STEC) E. coli strains as well as bacteria species that cross-react with the O157 antisera. All E. coli serotype O157 tested was positively identified while all other species, including the closely related O55 were not detected by the assay. Positive detection of E. coli O157:H7 was demonstrated when >10(2) CFU/ml was present in the samples. The capability of the assay to detect E. coli O157:H7 in raw milk and apple juice was demonstrated. As few as 1 CFU/ml was detected after 6 h of enrichment. These assays could be carried out entirely in sealed PCR tubes, enabling rapid and semiautomated detection of E. coli O157:H7 in food and environmental samples. Copyright 2001 Academic Press.

L4 ANSWER 4 OF 30 MEDLINE
ACCESSION NUMBER: 2001269761 MEDLINE
DOCUMENT NUMBER: 21148037 PubMed ID: 11249989
TITLE: Lipoprotein receptors: beacons to neurons?.
AUTHOR: Herz J
CORPORATE SOURCE: Department of Molecular Genetics, UT Southwestern, 5323 Harry Hines Boulevard, Dallas, TX 75390-9046, USA.. herz@UTSouthwestern.edu
SOURCE: TRENDS IN NEUROSCIENCES, (2001 Apr) 24 (4) 193-5. Ref: 12
Journal code: WEL; 7808616. ISSN: 0166-2236.
PUB. COUNTRY: England: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW LITERATURE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010529
Last Updated on STN: 20010529
Entered Medline: 20010521

AB Lipoprotein receptors were originally considered simply as cellular transporters for cholesterol and other lipids. This view is rapidly changing. Signaling functions have recently been recognized in several members of the low-density lipoprotein receptor gene family. These Apolipoprotein E receptors are highly expressed in the developing and in the mature nervous system, in which they regulate crucial developmental processes and might also participate in synaptic neurotransmission.

L4 ANSWER 5 OF 30 MEDLINE
ACCESSION NUMBER: 2001234556 MEDLINE
DOCUMENT NUMBER: 21113179 PubMed ID: 11160915
TITLE: Simultaneous A8344G heteroplasmy and mitochondrial DNA copy number quantification in myoclonus epilepsy and ragged-red fibers (MERRF) syndrome by a multiplex molecular beacon based real-time fluorescence PCR.

AUTHOR: Szuhai K; Ouweland J; Dirks R; Lemaitre M; Truffert J; Janssen G; Tanke H; Holme E; Maassen J; Raap A
CORPORATE SOURCE: Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands.
SOURCE: NUCLEIC ACIDS RESEARCH, (2001 Feb 1) 29 (3) E13. Journal code: O8L; 0411011. ISSN: 1362-4962.
PUB. COUNTRY: England: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010517
Last Updated on STN: 20010521
Entered Medline: 20010503

AB The association of a particular mitochondrial DNA (mtDNA) mutation with different clinical phenotypes is a well-known feature of mitochondrial diseases. A simple genotype-phenotype correlation has not been found between mutation load and disease expression. Tissue and intercellular mosaicism as well as mtDNA copy number are thought to be responsible for the different clinical phenotypes. As disease expression of mitochondrial tRNA mutations is mostly in postmitotic tissues, studies to elucidate disease mechanisms need to be performed on patient material. Heteroplasmy quantitation and copy number estimation using small patient biopsy samples has not been reported before, mainly due to technical restrictions. In order to resolve this problem, we have developed a robust assay that utilizes Molecular Beacons to accurately quantify heteroplasmy levels and determine mtDNA copy number in small samples carrying the A8344G tRNA(Lys) mutation. It provides the methodological basis to investigate the role of heteroplasmy and mtDNA copy number in determining the clinical phenotypes.

L4 ANSWER 6 OF 30 MEDLINE
ACCESSION NUMBER: 2000456015 MEDLINE
DOCUMENT NUMBER: 20441596 PubMed ID: 10987312
TITLE: In vivo imaging of proteolytic enzyme activity using a novel molecular reporter.
AUTHOR: Tung C H; Mahmood U; Bredow S; Weissleder R
CORPORATE SOURCE: Center for Molecular Imaging Research, Massachusetts General Hospital, Harvard Medical School, Charlestown 02129, USA.
CONTRACT NUMBER: R21-DK55713-01 (NIDDK)
SOURCE: CANCER RESEARCH, (2000 Sep 1) 60 (17) 4953-8. Journal code: CNF; 2984705R. ISSN: 0008-5472.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 20001005
Last Updated on STN: 20001005
Entered Medline: 20000928

AB The single biggest challenge facing in vivo imaging techniques is to develop biocompatible molecular beacons that are capable of specifically and accurately measuring in vivo targets at the protein, RNA, or DNA level. Our efforts have focused on developing activatable imaging probes to measure specific enzyme activities in vivo. Using cathepsin D as a model target protease, we synthesized a long-circulating, synthetic graft copolymer bearing near-infrared (NIR) fluorochromes positioned on cleavable substrate sequences. In its native state, the reporter probe was essentially nonfluorescent at 700 nm due to energy resonance transfer among the bound fluorochromes (quenching) but became brightly fluorescent when the latter were released by cathepsin D. NIR fluorescence signal activation was linear over at least 4 orders of magnitude and specific when compared with scrambled nonsense substrates. Using matched rodent tumor models implanted into nude mice expressing or lacking the targeted protease, it could be shown that the former generated sufficient NIR signal to be directly detectable and that the signal was significantly different compared with negative control tumors. The developed probes should find widespread applications for real-time in vivo imaging of a variety of clinically relevant proteases, for example, to detect

endogenous protease activity in disease, to monitor the efficacy of protease inhibitors, or to image transgene expression.

L4 ANSWER 7 OF 30 MEDLINE
ACCESSION NUMBER: 2000437409 MEDLINE
DOCUMENT NUMBER: 20381097 PubMed ID: 10921935
TITLE: Rapid identification of Candida dubliniensis using a species-specific molecular beacon.
AUTHOR: Park S; Wong M; Marras S A; Cross E W; Kiehn T E; Chaturvedi V; Tyagi S; Perlin D S
CORPORATE SOURCE: Public Health Research Institute, New York, NY 10016, USA.
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (2000 Aug) 38 (8) 2829-36.
Journal code: HSH; 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 20000928
Last Updated on STN: 20000928
Entered Medline: 20000920
AB Candida dubliniensis is an opportunistic fungal pathogen that has been linked to oral candidiasis in AIDS patients, although it has recently been isolated from other body sites. DNA sequence analysis of the internal transcribed spacer 2 (ITS2) region of rRNA genes from reference Candida strains was used to develop molecular beacon probes for rapid, high-fidelity identification of C. dubliniensis as well as C. albicans. Molecular beacons are small nucleic acid hairpin probes that brightly fluoresce when they are bound to their targets and have a significant advantage over conventional nucleic acid probes because they exhibit a higher degree of specificity with better signal-to-noise ratios. When applied to an unknown collection of 23 strains that largely contained C. albicans and a smaller amount of C. dubliniensis, the species-specific probes were 100% accurate in identifying both species following PCR amplification of the ITS2 region. The results obtained with the molecular beacons were independently verified by random amplified polymorphic DNA analysis-based genotyping and by restriction enzyme analysis with enzymes BsmAI and NspBII, which cleave recognition sequences within the ITS2 regions of C. dubliniensis and C. albicans, respectively. Molecular beacons are promising new probes for the rapid detection of Candida species.

L4 ANSWER 8 OF 30 MEDLINE
ACCESSION NUMBER: 2001033545 MEDLINE
DOCUMENT NUMBER: 20527879 PubMed ID: 11078442
TITLE: Beacon: a novel gene involved in the regulation of energy balance.
AUTHOR: Collier G R; McMillan J S; Windmill K; Walder K; Tenne-Brown J; de Silva A; Trevaskis J; Jones S; Morton G J; Lee S; Augert G; Civitarese A; Zimmet P Z
CORPORATE SOURCE: Metabolic Research Unit, School of Health Sciences, Deakin University, Geelong, Victoria, Australia..
beacon@deakin.edu.au
SOURCE: DIABETES, (2000 Nov) 49 (11) 1766-71.
Journal code: E8X. ISSN: 0012-1797.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200011
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20001130
AB The hypothalamus plays a major role in the control of energy balance via the coordination of several neuropeptides and their receptors. We used a unique polygenic animal model of obesity, Psammomys obesus, and performed differential display polymerase chain reaction on hypothalamic mRNA samples to identify novel genes involved in obesity. In this study, we describe a novel gene that encodes a small protein we

have termed "beacon." Beacon mRNA gene expression in the hypothalamus was positively correlated with percentage of body fat. Intracerebroventricular infusion of beacon resulted in a dose-dependent increase in food intake and body weight and an increase in hypothalamic expression of neuropeptide Y (NPY). Simultaneous infusion of beacon and NPY significantly potentiated the orexigenic response and resulted in rapid body weight gain. These data suggest a role for beacon in the regulation of energy balance and body weight homeostasis that may be mediated, at least in part, through the NPY pathway.

L4 ANSWER 9 OF 30 MEDLINE
ACCESSION NUMBER: 2001190720 MEDLINE
DOCUMENT NUMBER: 21017195 PubMed ID: 11126133
TITLE: Semiautomated clone verification by real-time PCR using molecular beacons.
AUTHOR: van Schie R C; Marras S A; Conroy J M; Nowak N J; Catanese J J; de Jong P J
CORPORATE SOURCE: Roswell Park Cancer Institute, Buffalo, NY, USA.. vanschie@roswellpark.org
CONTRACT NUMBER: 1R01 HG01165-05 (NHGRI)
HL-43521 (NHLBI)
SOURCE: BIOTECHNIQUES, (2000 Dec) 29 (6) 1296-300, 1302-4, 1306 passim.
Journal code: AN3; 8306785. ISSN: 0736-6205.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200104
ENTRY DATE: Entered STN: 20010410
Last Updated on STN: 20010410
Entered Medline: 20010405
AB Conventional, high-throughput PCR analysis of common elements utilizing numerous primer sets and template DNA requires multiple rounds of PCR to ensure optimal conditions. Laborious gel electrophoresis and staining is then necessary to visualize amplification products. We propose novel multicolor molecular beacons, to establish a high-throughput, PCR-based sequence tagged site (STS) detection system that swiftly and accurately confirms marker content in template containing common repeat elements. A simple, one-tube, real-time PCR assay system was developed to specifically detect regions containing CA and GATA repeats. Ninety-six samples can be confirmed for marker content in a closed-tube format in 3 h, eliminating product confirmation on agarose gels and avoiding crossover contamination. Multiple STSs can be detected simultaneously in the same reaction tube by utilizing molecular beacons labeled with multicolor fluorophores. Template DNA from 260 RPCI-11 bacterial artificial chromosome (BAC) clones was examined for the presence of CA and/or GATA repeats using molecular beacon PCR and compared with conventional PCR results of the same clones. Of the 205 clones containing CA and GATA repeats, we were able to identify 129 clones (CA, n = 99; GATA, n = 30) by using molecular beacons and only 121 clones (CA, n = 92; GATA, n = 29) by conventional PCR amplification. As anticipated, 55 clones that contained sequences other than CA or GATA failed molecular beacon detection. Molecular beacon PCR, employing beacons specific for tandem repeat elements, provides a fast, accurate, and sensitive multiplex detection assay that will expedite verification of marker content in a multitude of template containing these repeats.

L4 ANSWER 10 OF 30 MEDLINE
ACCESSION NUMBER: 2001123943 MEDLINE
DOCUMENT NUMBER: 20553881 PubMed ID: 11101699
TITLE: Real-time PCR using molecular beacons for accurate detection of the Y chromosome in single human blastomeres.
AUTHOR: Pierce K E; Rice J E; Sanchez J A; Brenner C; Wangh L J
CORPORATE SOURCE: Department of Biology, Brandeis University, Waltham, MA 02454-9110, USA.
SOURCE: MOLECULAR HUMAN REPRODUCTION, (2000 Dec) 6 (12) 1155-64.

Journal code: CWO. ISSN: 1360-9947.
PUB. COUNTRY: ENGLAND: United Kingdom
(EVALUATION STUDIES)
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010222
AB We describe a highly accurate method for determining the sex of human embryos via real-time polymerase chain reaction (PCR) amplification of highly-conserved, moderately-repeated sequences within the TSPY genes on the Y chromosome and the U2 genes on chromosome 17. Individual male lymphocytes, female lymphocytes, and blastomeres from donated cleavage-stage embryos were lysed prior to PCR using an optimized buffer containing proteinase K. Molecular beacons, a new type of fluorescent probe, were used to detect and quantify accumulating amplicons during each cycle of PCR carried out in closed tubes. The present work is part of an ongoing study to construct and implement a new, convenient and reliable system of preimplantation genetic diagnosis (PGD).

L4 ANSWER 11 OF 30 MEDLINE
ACCESSION NUMBER: 2000434818 MEDLINE
DOCUMENT NUMBER: 20370321 PubMed ID: 10914649
TITLE: Molecular beacon polymerase chain reaction detection of Escherichia coli O157:H7 in milk.
AUTHOR: McKillip J L; Drake M
CORPORATE SOURCE: Department of Food Science and Technology, Southeast Dairy Foods Research Center, Mississippi State University, Mississippi State 39762, USA.
SOURCE: JOURNAL OF FOOD PROTECTION, (2000 Jul) 63 (7) 855-9.
Journal code: C48; 7703944. ISSN: 0362-028X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 20000928
Last Updated on STN: 20000928
Entered Medline: 20000921
AB A fluorescently labeled oligonucleotide probe (molecular beacon) was applied to detect Escherichia coli O157:H7 in artificially contaminated skim milk during polymerase chain reaction (PCR) amplification of extracted DNA. The probe was designed to hybridize with a region of the slt-II gene coding for the A subunit and to fluoresce when the hairpin-stem conformation was linearized upon hybridization to the target sequence. The molecular beacon was incorporated into PCR reactions containing DNA extracted from artificially contaminated skim milk. The degree of fluorescence was monitored in PCR reactions containing 10(3), 10(5), and 10(7) CFU of E. coli O157:H7 per ml and was found to correlate with the amount of template in each reaction. Fluorescence significantly increased above background levels by cycle 8, 14, or 14 in reactions containing DNA from the 10(7)-, 10(5)-, or 10(3)-CFU/ml template, respectively ($P < 0.05$). Molecular beacon PCR demonstrated positive results more rapidly than traditional agarose gel electrophoresis analysis of PCR products. Use of molecular beacons allows real-time monitoring of PCR reactions, and the closed-tube format allows simultaneous detection and confirmation of target amplicons without the need for agarose gel electrophoresis and/or Southern blotting. This is the first report of a stem-and-loop molecular beacon being applied for direct detection of a pathogen in food.

L4 ANSWER 12 OF 30 MEDLINE
ACCESSION NUMBER: 2000232585 MEDLINE
DOCUMENT NUMBER: 20232585 PubMed ID: 10769752
TITLE: Homogeneous scoring of single-nucleotide polymorphisms: comparison of the 5'-nuclease TaqMan assay and Molecular Beacon probes.
AUTHOR: Tapp I; Malmberg L; Rennel E; Wik M; Syvanen A C

CORPORATE SOURCE: Uppsala University, Sweden.
SOURCE: BIOTECHNIQUES, (2000 Apr) 28 (4) 732-8.
Journal code: AN3; 8306785. ISSN: 0736-6205.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200006
ENTRY DATE: Entered STN: 20000622
Last Updated on STN: 20000622
Entered Medline: 20000609

AB Homogeneous assays based on real-time fluorescence monitoring during PCR are relevant alternatives for large-scale genotyping of single-nucleotide polymorphisms (SNPs). We compared the performance of the homogeneous TaqMan 5'-nuclease assay and the Molecular Beacon assay using three SNPs in the human estrogen receptor gene as targets. When analyzing a panel of 90 DNA samples, both assays yielded a comparable power of discrimination between the genotypes of a C-to-T transition in codon 10 and a G-to-A transition in codon 594 of the estrogen receptor gene. The Molecular Beacon probes distinguished better than the TaqMan probes between homozygous and heterozygous genotypes of a C-to-G transversion in codon 325. The sensitivity of detecting one allele, present as a minority in a mixed sample, varied between the SNPs and was similar for both assays. With the Molecular Beacon assay, the measured signal ratios were proportional to the amount of the minor allele over a wider range than with the TaqMan assay at all three SNPs.

L4 ANSWER 13 OF 30 MEDLINE
ACCESSION NUMBER: 2000241622 MEDLINE
DOCUMENT NUMBER: 20241622 PubMed ID: 10780710
TITLE: Global distribution of the CCR2-64I/CCR5-59653T HIV-1 disease-protective haplotype.
AUTHOR: Martinson J J; Hong L; Karanicolas R; Moore J P; Kostrikis L G
CORPORATE SOURCE: Department of Biological Anthropology, Oxford University, UK.
CONTRACT NUMBER: AI41420 (NIAID)
RO1 AI43868 (NIAID)
SOURCE: AIDS, (2000 Mar 31) 14 (5) 483-9.
Journal code: AID; 8710219. ISSN: 0269-9370.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200006
ENTRY DATE: Entered STN: 20000629
Last Updated on STN: 20000629
Entered Medline: 20000616

AB OBJECTIVES: Several natural polymorphisms in the genes for the human CC-chemokine receptors CCR5 and CCR2 are associated with HIV-1 disease. The CCR2-64I genetic variant [a G to A substitution resulting in a valine (V) to isoleucine (I) change at position 64] is in strong linkage disequilibrium with a mutation within the CCR5 regulatory region (CCR5-59653T). Individuals with two CCR2-64I alleles are not resistant to sexual transmission of HIV-1, but progress significantly more slowly to HIV-1 disease. It is therefore important to determine the global distributions of CCR2-64I and CCR5-59653T genetic variants and define the degree of linkage between them. DESIGN AND METHODS: We have developed molecular beacon-based, real-time PCR allele discrimination assays for all three chemokine receptor mutations, and used these spectral genotyping assays to genotype 3923 individuals from a globally distributed set of 53 populations. RESULTS: CCR2-64I and CCR5-59653T genetic variants are found in almost all populations studied: their allele frequencies are greatest (approximately 35%) in Africa and Asia but decrease in Northern Europe. We confirm that CCR2-64I is in strong linkage disequilibrium with CCR5-59653T (96.92% of individuals had the same genotype for both CCR2-64I and CCR5-59653T polymorphisms). CONCLUSIONS: The greater geographical distribution of the CCR2-64I/CCR5-59653T haplotype compared with that of CCR5-delta32 suggests that it is a much older mutation whose origin predates the dispersal of modern humans.

L4 ANSWER 14 OF 30 MEDLINE
ACCESSION NUMBER: 2000385482 MEDLINE
DOCUMENT NUMBER: 20345607 PubMed ID: 10886366
TITLE: Molecular beacon aptamer fluoresces in the presence of Tat protein of HIV-1.
COMMENT: Erratum in: Genes Cells 2000 Jun;5(6):423
AUTHOR: Yamamoto R; Baba T; Kumar P K
CORPORATE SOURCE: National Institute of Bioscience and Human Technology, University of Tsukuba, Tsukuba 305-8572, Japan.
SOURCE: GENES TO CELLS, (2000 May) 5 (5) 389-96.
Journal code: CUF; 9607379. ISSN: 1356-9597.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000818
Last Updated on STN: 20001019
Entered Medline: 20000808
AB BACKGROUND: We reported an aptamer, RNATat that binds to the Tat protein of HIV with two orders of magnitude greater (133-fold) affinity over the TAR RNA of HIV-1 and specifically inhibits the Tat-dependent trans-activation of transcription, both in vitro and in vivo (demonstrated in the accompanying article, Yamamoto et al., this issue pp. 371-388). We now report the use of aptamer-derived oligomers to analyze the Tat of HIV and the possible applications of such constructs in the field of biosensors. RESULTS: To make new molecular beacon, we constructed two RNA oligomers that derived from RNATat. To one of the split RNA oligomers that forms a hairpin structure, the fluorophore and quencher were attached at the 5'- and 3'-ends, respectively. Specifically in the presence of Tat or its peptides, but not in the presence of other RNA binding proteins, the two oligomers undergo a conformational change to form a duplex that leads to relieving of fluorophore from the quencher, and thus a significant enhancement of the fluorescence of fluorescein was observed. CONCLUSION: A novel strategy for exploiting aptamers in the analysis of Tat (analyte) has been described. A similar strategy could be used to study other analytes such as proteins and small molecules. In addition, the molecular beacon aptamer requires half the length of target sequence (eight nucleotides) in comparison with molecular beacons. Thus, it is conceivable that we could insert an analyte-binding site into molecular beacons to convert them to signalling beacons.

L4 ANSWER 15 OF 30 MEDLINE
ACCESSION NUMBER: 2000263285 MEDLINE
DOCUMENT NUMBER: 20263285 PubMed ID: 10805535
TITLE: Molecular beacons: a real-time polymerase chain reaction assay for detecting Salmonella.
AUTHOR: Chen W; Martinez G; Mulchandani A
CORPORATE SOURCE: Department of Chemical and Environmental Engineering, University of California, Riverside 92521, USA..
Wilfred@engr.ucr.edu
SOURCE: ANALYTICAL BIOCHEMISTRY, (2000 Apr 10) 280 (1) 166-72.
Journal code: 4NK; 0370535. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200006
ENTRY DATE: Entered STN: 20000714
Last Updated on STN: 20000714
Entered Medline: 20000630
AB Molecular beacons are oligonucleotide probes that become fluorescent upon hybridization. We developed a real-time PCR assay to detect the presence of Salmonella species using these fluorogenic reporter molecules. A 122-base-pair section of the himA was used as the amplification target. Molecular beacons were designed to recognize a 16-base-pair region on the amplicon. As few as 2 colony-forming unit (CFU) per PCR reaction could be detected. We also

demonstrated the ability of the molecular beacons to discriminate between amplicons obtained from similar species such as Escherichia coli and Citrobacter freundii in real-time PCR assays. These assays could be carried out entirely in sealed PCR tubes, enabling fast and direct detection of Salmonella in a semiautomated format.

L4 ANSWER 16 OF 30 MEDLINE
ACCESSION NUMBER: 2000068683 MEDLINE
DOCUMENT NUMBER: 20068683 PubMed ID: 10602730
TITLE: Genotypic analysis of Mycobacterium tuberculosis in two distinct populations using molecular beacons: implications for rapid susceptibility testing.
AUTHOR: Piatek A S; Telenti A; Murray M R; El-Hajj H; Jacobs W R Jr; Kramer F R; Alland D
CORPORATE SOURCE: Division of Infectious Diseases, Department of Medicine, Montefiore Medical Center, Bronx, New York 10467, USA.
CONTRACT NUMBER: 5T32 (NIAID)
AI37015 (NIAID)
AI45244
+
SOURCE: ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, (2000 Jan) 44 (1) 103-10.
Journal code: 6HK; 0315061. ISSN: 0066-4804.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200001
ENTRY DATE: Entered STN: 20000124
Last Updated on STN: 20000124
Entered Medline: 20000111
AB Past genotypic studies of Mycobacterium tuberculosis may have incorrectly estimated the importance of specific drug resistance mutations due to a number of sampling biases including an overrepresentation of multidrug-resistant (MDR) isolates. An accurate assessment of resistance mutations is crucial for understanding basic resistance mechanisms and designing genotypic drug resistance assays. We developed a rapid closed-tube PCR assay using fluorogenic reporter molecules called molecular beacons to detect reportedly common M. tuberculosis mutations associated with resistance to isoniazid and rifampin. The assay was used in a comparative genotypic investigation of two different study populations to determine whether these known mutations account for most cases of clinical drug resistance. We analyzed samples from a reference laboratory in Madrid, Spain, which receives an overrepresentation of MDR isolates similar to prior studies and from a community medical center in New York where almost all of the resistant isolates and an equal number of susceptible controls were available. The ability of the molecular beacon assay to predict resistance to isoniazid and rifampin was also assessed. The overall sensitivity and specificity of the assay for isoniazid resistance were 85 and 100%, respectively, and those for rifampin resistance were 98 and 100%, respectively. Rifampin resistance mutations were detected equally well in isolates from both study populations; however, isoniazid resistance mutations were detected in 94% of the isolates from Madrid but in only 76% of the isolates from New York ($P = 0.02$). In New York, isoniazid resistance mutations were significantly more common in the MDR isolates (94%) than in single-drug-resistant isolates (44%; $P < 0.001$). No association between previously described mutations in the kasA gene and isoniazid resistance was found. The first mutations that cause isoniazid resistance may often occur in sequences that have not been commonly associated with isoniazid resistance, possibly in other as yet uncharacterized genes. The molecular beacon assay was simple, rapid, and highly sensitive for the detection of rifampin-resistant M. tuberculosis isolates and for the detection of isoniazid resistance in MDR isolates.

L4 ANSWER 17 OF 30 MEDLINE
ACCESSION NUMBER: 2000508231 MEDLINE
DOCUMENT NUMBER: 20512594 PubMed ID: 11058144
TITLE: PCR hot start using primers with the structure of molecular beacons (hairpin-like structure).

AUTHOR: Kaboev O K; Luchkina L A; Tret'iakov A N; Bahrmand A R
CORPORATE SOURCE: St Petersburg Nuclear Physics Institute, Russian Academy of Science, Gatchina 188350, Russia and Tehran Pasteur Institute, Iran.. kaboev@omrb.pnpi.spb.ru
SOURCE: NUCLEIC ACIDS RESEARCH, (2000 Nov 1) 28 (21) E94. Journal code: O8L; 0411011. ISSN: 1362-4962.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200011
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20010521
Entered Medline: 20001116

AB A new technique of PCR hot start using oligonucleotide primers with a stem-loop structure is developed here. The molecular beacon oligonucleotide structure without any chromophore addition to the ends was used. The 3'-end sequence of the primers was complementary to the target and five or six nucleotides complementary to the 3'-end were added to the 5'-end. During preparation of the reaction mixture and initial heating, the oligonucleotide has a stem-loop structure and cannot serve as an effective primer for DNA polymerase. After heating to the annealing temperature it acquires a linear structure and primer extension can begin.

L4 ANSWER 18 OF 30 MEDLINE
ACCESSION NUMBER: 2000091150 MEDLINE
DOCUMENT NUMBER: 20091150 PubMed ID: 10625399
TITLE: Screening unlabeled DNA targets with randomly ordered fiber-optic gene arrays.
AUTHOR: Steemers F J; Ferguson J A; Walt D R
CORPORATE SOURCE: The Max Tishler Laboratory for Organic Chemistry, Department of Chemistry, Tufts University, Medford, MA.
CONTRACT NUMBER: GM48142 (NIGMS)
SOURCE: NATURE BIOTECHNOLOGY, (2000 Jan) 18 (1) 91-4. Journal code: CQ3; 9604648. ISSN: 1087-0156.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200002
ENTRY DATE: Entered STN: 20000314
Last Updated on STN: 20000314
Entered Medline: 20000229

AB We have developed a randomly ordered fiber-optic gene array for rapid, parallel detection of unlabeled DNA targets with surface immobilized molecular beacons (MB) that undergo a conformational change accompanied by a fluorescence change in the presence of a complementary DNA target. Microarrays are prepared by randomly distributing MB-functionalized 3-microm diameter microspheres in an array of wells etched in a 500-microm diameter optical imaging fiber. Using several MBs, each designed to recognize a different target, we demonstrate the selective detection of genomic cystic fibrosis related targets. Positional registration and fluorescence response monitoring of the microspheres was performed using an optical encoding scheme and an imaging fluorescence microscope system.

L4 ANSWER 19 OF 30 MEDLINE
ACCESSION NUMBER: 2000189986 MEDLINE
DOCUMENT NUMBER: 20189986 PubMed ID: 10722789
TITLE: Symmetric vs asymmetric PCR and molecular beacon probe in the detection of a target gene of adenovirus.
AUTHOR: Poddar S K
CORPORATE SOURCE: Department of Pediatrics, Division of Infectious Diseases and Pediatric Pharmacology Research Unit (PPRU), La Jolla, CA 92093-0808, USA.. poddar@nhrc.navy.mil
SOURCE: MOLECULAR AND CELLULAR PROBES, (2000 Feb) 14 (1) 25-32. Journal code: NG9; 8709751. ISSN: 0890-8508.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200005
ENTRY DATE: Entered STN: 20000518
Last Updated on STN: 20000518
Entered Medline: 20000505

AB A DNA fragment (307 bp) from the conserved region of an adenovirus gene (hexon) was amplified by symmetric and by asymmetric polymerase chain reaction (PCR). Two amplifications, one in the absence other in the presence of a molecular beacon probe were conducted by both symmetric and asymmetric PCR. The probe sequence was complementary to an internal segment of the amplified fragment. The product amplified in the absence and presence of the probe was detected by agarose gel and fluorescence analysis, respectively. A symmetric PCR results in exponentially grown double stranded DNA. An asymmetric PCR generates one of the strands by linear amplification and a fraction of its total product as double-stranded DNA limited by the concentration ratio of the primers used. Thus asymmetric PCR provided lower intensity signal hence less sensitivity than symmetric PCR by agarose gel analysis as expected. However, signal from a beacon probe based PCR assay is generated only from the probe fraction that hybridizes successfully competing against the strand complementary to the target strand of the product generated by PCR. The symmetric PCR has so far been used for the molecular beacon based fluorescent signal detection. The present study compared the level of fluorescent signal detectable from a symmetric PCR with that from an asymmetric PCR. The fluorescent data analysis demonstrated that a significant higher level of fluorescent signal hence higher sensitivity of detection is obtainable using asymmetric PCR than symmetric PCR performed in presence of the molecular beacon probe.

L4 ANSWER 20 OF 30 MEDLINE
ACCESSION NUMBER: 1999272704 MEDLINE
DOCUMENT NUMBER: 99272704 PubMed ID: 10339598
TITLE: Multiplex detection of four pathogenic retroviruses using molecular beacons.
AUTHOR: Vet J A; Majithia A R; Marras S A; Tyagi S; Dube S; Poiesz B J; Kramer F R
CORPORATE SOURCE: Department of Molecular Genetics, Public Health Research Institute, 455 First Avenue, New York, NY 10016, USA.
CONTRACT NUMBER: HB-67131 (NHLBI)
HL-43521 (NHLBI)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 May 25) 96 (11) 6394-9.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990712
Last Updated on STN: 19990712
Entered Medline: 19990624

AB We describe a multiplex nucleic acid assay that identifies and determines the abundance of four different pathogenic retroviruses (HIV-1, HIV-2, and human T-lymphotrophic virus types I and II). Retroviral DNA sequences are amplified in a single, sealed tube by simultaneous PCR assays, and the resulting amplicons are detected in real time by the hybridization of four differently colored, amplicon-specific molecular beacons. The color of the fluorescence generated in the course of amplification identifies which retroviruses are present, and the number of thermal cycles required for the intensity of each color to rise significantly above background provides an accurate measure of the number of copies of each retroviral sequence that were present originally in the sample. Fewer than 10 retroviral genomes can be detected. Moreover, 10 copies of a rare retrovirus can be detected in the presence of 100, 000 copies of an abundant retrovirus. Ninety-six samples can be analyzed in 3 hr on a single plate, and the use of a closed-tube format eliminates crossover contamination. Utilizing previously well characterized clinical samples,

we demonstrate that each of the pathogenic retroviruses can be identified correctly and no false positives occur. This assay enables the rapid and reliable screening of donated blood and transplantable tissues.

L4 ANSWER 21 OF 30 MEDLINE
ACCESSION NUMBER: 1999262764 MEDLINE
DOCUMENT NUMBER: 99262764 PubMed ID: 10325321
TITLE: Molecular epidemiologic evaluation of transmissibility and virulence of Mycobacterium tuberculosis.
AUTHOR: Rhee J T; Piatek A S; Small P M; Harris L M; Chaparro S V; Kramer F R; Alland D
CORPORATE SOURCE: Division of Epidemiology, Department of Health Research and Policy, Stanford University School of Medicine 94305, USA.
CONTRACT NUMBER: AI-43268 (NIAID)
AJ-23238
HL-43521 (NHLBI)
SOURCE: JOURNAL OF CLINICAL MICROBIOLOGY, (1999 Jun) 37 (6) 1764-70.
Journal code: HSH; 7505564. ISSN: 0095-1137.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199906
ENTRY DATE: Entered STN: 19990712
Last Updated on STN: 19990712
Entered Medline: 19990623
AB Discovery of genotypic markers associated with increased transmissibility in Mycobacterium tuberculosis would represent an important step in advancing mycobacterial virulence studies. M. tuberculosis strains may be classified into one of three genotypes on the basis of the presence of specific nucleotide substitutions in codon 463 of the katG gene (katG-463) and codon 95 of the gyrA gene (gyrA-95). It has previously been reported that two of these three genotypes are associated with increased IS6110-based clustering, a potential proxy of virulence. We designed a case-control analysis of U.S.-born patients with tuberculosis in San Francisco, Calif., between 1991 and 1997 to investigate associations between katG-463 and gyrA-95 genotypes and epidemiologically determined measures of strain-specific infectivity and pathogenicity and IS6110-based clustering status. We used a new class of molecular probes called molecular beacons to genotype the isolates rapidly. Infectivity was defined as the propensity of isolates to cause tuberculin skin test conversions among named contacts, and pathogenicity was defined as their propensity to cause active disease among named contacts. The molecular beacon assay was a simple and reproducible method for the detection of known single nucleotide polymorphisms in large numbers of clinical M. tuberculosis isolates. The results showed that no genotype of the katG-463- and gyrA-95-based classification system was associated with increased infectivity and pathogenicity or with increased IS6110-based clustering in San Francisco during the study period. We speculate that molecular epidemiologic studies investigating clinically relevant outcomes may contribute to the knowledge of the significance of laboratory-derived virulence factors in the propagation of tuberculosis in human communities.

L4 ANSWER 22 OF 30 MEDLINE
ACCESSION NUMBER: 2000009812 MEDLINE
DOCUMENT NUMBER: 20009812 PubMed ID: 10541565
TITLE: Analysis of gene expression in single oocytes and embryos by real-time rapid cycle fluorescence monitored RT-PCR.
AUTHOR: Steuerwald N; Cohen J; Herrera R J; Brenner C A
CORPORATE SOURCE: Gamete and Embryo Research Laboratory, Institute for Reproductive Medicine and Science of Saint Barnabas, West Orange, New Jersey, 07052, USA.
SOURCE: MOLECULAR HUMAN REPRODUCTION, (1999 Nov) 5 (11) 1034-9.
Journal code: CWO; 9513710. ISSN: 1360-9947.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 199911
ENTRY DATE: Entered STN: 20000113
Last Updated on STN: 20000113
Entered Medline: 19991130

AB Rapid cycle DNA amplification is a refinement of the polymerase chain reaction (PCR) method that permits increased product specificity while reducing amplification time by an order of magnitude. Combined with the use of micro volume capillaries, minute samples can be examined by this technique. Thus, this approach is ideally suited to the analysis of gene expression in individual cells. As the current understanding of early developmental processes is still rudimentary, further characterization of transcription in single oocytes and embryos may provide additional insight into the molecular mechanisms directing these events. In this study, we examined the suitability of fluorescence monitored reverse transcription (RT)-PCR for the study of gene expression during oogenesis and embryogenesis using transcripts of the housekeeping gene, beta-actin, as an experimental model. Product accumulation was monitored by either the double-stranded DNA dye SYBR Green I or sequence-dependent hybridization of reporter molecules called molecular beacons. Dyes bind generically and are economical to use. However, both specific and non-specific products are labelled. Hybridization probes permit very specific and sensitive target recognition but they can be costly to manufacture. Once molecular markers indicative of optimal development are identified, this technology could be used in a clinical in-vitro fertilization laboratory as a diagnostic tool.

L4 ANSWER 23 OF 30 MEDLINE
ACCESSION NUMBER: 1999157576 MEDLINE
DOCUMENT NUMBER: 99157576 PubMed ID: 10027986
TITLE: Differential expression of 10 sigma factor genes in Mycobacterium tuberculosis.
AUTHOR: Manganelli R; Dubnau E; Tyagi S; Kramer F R; Smith I
CORPORATE SOURCE: Department of Microbiology, Public Health Research Institute, New York, NY 10016, USA.
CONTRACT NUMBER: GM-19693 (NIGMS)
HL-43521 (NHLBI)
SOURCE: MOLECULAR MICROBIOLOGY, (1999 Jan) 31 (2) 715-24.
Journal code: MOM; 8712028. ISSN: 0950-382X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199904
ENTRY DATE: Entered STN: 19990511
Last Updated on STN: 19990511
Entered Medline: 19990429

AB The ability of Mycobacterium tuberculosis to adapt to different environments in the infected host is essential for its pathogenicity. Consequently, this organism must be able to modulate gene expression to respond to the changing conditions it encounters during infection. In this paper we begin a comprehensive study of M. tuberculosis gene regulation, characterizing the transcript levels of 10 of its 13 putative sigma factor genes. We developed a real-time RT-PCR assay using a family of novel fluorescent probes called molecular beacons to quantitatively measure the different mRNAs. Three sigma factor genes were identified that have increased mRNA levels after heat shock, two of which also responded to detergent stress. In addition, we also identified a sigma factor gene whose mRNA increased after mild cold shock and a second that responded to conditions of low aeration.

L4 ANSWER 24 OF 30 MEDLINE
ACCESSION NUMBER: 1999435243 MEDLINE
DOCUMENT NUMBER: 99435243 PubMed ID: 10507409
TITLE: Detection of adenovirus using PCR and molecular beacon.
AUTHOR: Poddar S K
CORPORATE SOURCE: Department of Pediatrics, University of California at San Diego, La Jolla 92093-0808, USA.
SOURCE: JOURNAL OF VIROLOGICAL METHODS, (1999 Sep) 82 (1) 19-26.

Journal code: HQR; 8005839. ISSN: 0166-0934.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199911
ENTRY DATE: Entered STN: 20000111
Last Updated on STN: 20000111
Entered Medline: 19991123

AB The polymerase chain reaction (PCR) and a molecular beacon probe were used for the detection of Adenovirus. A 307 bp DNA fragment from a conserved region of the hexon gene was amplified. The specific molecular beacon was characterized with respect to its efficiency of quenching, and signal to noise ratio by spectrofluorometric analysis of its hybridization with virus specific complementary single stranded oligonucleotide target. Amplification was carried out in the presence of the molecular beacon probe, and the amplified target was detected by measurement of fluorescence signal in the post PCR sample. Separately, a 32P-labeled linear probe (having the same sequence as that of molecular beacon probe) was liquid-phase hybridized with the product of PCR performed in the absence of the molecular beacon. The virus specific target was then detected by electrophoresis of the hybridized product in a nondenaturing polyacrylamide gel and subsequent autoradiographic analysis. The detection limit of adenovirus by PCR in the presence of the molecular beacon probe was found to be similar to that obtained by labeled linear probe hybridization following PCR.

L4 ANSWER 25 OF 30 MEDLINE
ACCESSION NUMBER: 1998213743 MEDLINE
DOCUMENT NUMBER: 98213743 PubMed ID: 9547273
TITLE: Molecular beacon probes combined with amplification by NASBA enable homogeneous, real-time detection of RNA.
AUTHOR: Leone G; van Schijndel H; van Gemen B; Kramer F R; Schoen C D
CORPORATE SOURCE: DLO Research Institute for Plant Protection (IPO-DLO), PO Box 9060, 6700 GW Wageningen, The Netherlands.. leone@ipo.dlo.nl
SOURCE: NUCLEIC ACIDS RESEARCH, (1998 May 1) 26 (9) 2150-5.
Journal code: O8L; 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199806
ENTRY DATE: Entered STN: 19980625
Last Updated on STN: 19980625
Entered Medline: 19980616

AB Molecular beacon probes can be employed in a NASBA amplicon detection system to generate a specific fluorescent signal concomitantly with amplification. A molecular beacon, designed to hybridize within the target sequence, was introduced into NASBA reactions that amplify the genomic RNA of potato leafroll virus (PLRV). During amplification, the probe anneals to the antisense RNA amplicon generated by NASBA, producing a specific fluorescent signal that can be monitored in real-time. The assay is rapid, sensitive and specific. As RNA amplification and detection can be carried out in unopened vessels, it minimizes the risk of carry-over contaminations. Robustness has been verified on real-world samples. This homogeneous assay, called AmpliDet RNA, is a significant improvement over current detection methods for NASBA amplicons and is suitable for one-tube applications ranging from high-throughput diagnostics to in vivo studies of biological activities.

L4 ANSWER 26 OF 30 MEDLINE
ACCESSION NUMBER: 1998171840 MEDLINE
DOCUMENT NUMBER: 98171840 PubMed ID: 9510851
TITLE: Molecular beacons: a new approach for semiautomated mutation analysis.
AUTHOR: Giesendorf B A; Vet J A; Tyagi S; Mensink E J; Trijbels F J; Blom H J

CORPORATE SOURCE: University Hospital Nijmegen, Department of Pediatrics, The Netherlands.. B.Giesendorf@ckslkn.azn.nl
CONTRACT NUMBER: HL-43521 (NHLBI)
SOURCE: CLINICAL CHEMISTRY, (1998 Mar) 44 (3) 482-6.
Journal code: DBZ; 9421549. ISSN: 0009-9147.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980416
Last Updated on STN: 19980416
Entered Medline: 19980407

AB Molecular beacons are oligonucleotide probes that become fluorescent upon hybridization. We designed molecular beacons to detect a point mutation in the methylenetetrahydrofolate reductase (MTHFR) gene, a mutation that has been related to an increased risk for cardiovascular disease and neural tube defects. The application of molecular beacons enables fast, semiautomated, accurate mutation detection. Moreover, the procedure is performed in a closed tube system, thereby avoiding carryover contamination. We believe these probes will find their way into nucleic acid research and diagnostics.

L4 ANSWER 27 OF 30 MEDLINE
ACCESSION NUMBER: 1998216568 MEDLINE
DOCUMENT NUMBER: 98216568 PubMed ID: 9555727
TITLE: Molecular beacon sequence analysis for detecting drug resistance in Mycobacterium tuberculosis.
COMMENT: Comment in: Nat Biotechnol. 1998 Apr;16(4):331
AUTHOR: Piatek A S; Tyagi S; Pol A C; Telenti A; Miller L P; Kramer F R; Alland D
CORPORATE SOURCE: Department of Medicine, Montefiore Medical Center, Bronx, NY 10467, USA.
CONTRACT NUMBER: AI 37015 (NIAID)
AI 45244 (NIAID)
HL 43521 (NHLBI)
SOURCE: NATURE BIOTECHNOLOGY, (1998 Apr) 16 (4) 359-63.
Journal code: CQ3; 9604648. ISSN: 1087-0156.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980611
Last Updated on STN: 19980611
Entered Medline: 19980529

AB We developed a new approach to DNA sequence analysis that uses fluorogenic reporter molecules--molecular beacons--and demonstrated their ability to discriminate alleles in real-time PCR assays of genomic DNA. A set of overlapping molecular beacons was used to analyze an 81-bp region of the Mycobacterium tuberculosis rpoB gene for mutations that confer resistance to the antibiotic rifampin. In a blinded study of 52 rifampin-resistant and 23 rifampin-susceptible clinical isolates, this method correctly detected mutations in all of the resistant strains and in none of the susceptible strains. The assay was carried out entirely in sealed PCR tubes and was simple to perform and interpret. This approach can be used to analyze any DNA sequence of moderate length with single base pair accuracy.

L4 ANSWER 28 OF 30 MEDLINE
ACCESSION NUMBER: 1998396286 MEDLINE
DOCUMENT NUMBER: 98396286 PubMed ID: 9727198
TITLE: PNA molecular beacons for rapid detection of PCR amplicons.
AUTHOR: Ortiz E; Estrada G; Lizardi P M
CORPORATE SOURCE: Department of Molecular Recognition and Structural Biology, Universidad Nacional Autonoma de Mexico, Cuernavaca, Morelos, Mexico.
SOURCE: MOLECULAR AND CELLULAR PROBES, (1998 Aug) 12 (4) 219-26.
Journal code: NG9; 8709751. ISSN: 0890-8508.

PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY DATE: Entered STN: 19990106
Last Updated on STN: 19990106
Entered Medline: 19981124

AB The authors have developed a method for rapid detection of polymerase chain reaction (PCR) amplicons based on surface immobilized PNA-DNA hybrid probes ('molecular beacons') that undergo a fluorescent-linked conformational change in the presence of a complementary DNA target. Amplicons can be detected by simply adding a PCR reaction to a microtitre-well containing the previously immobilized probe, and reading the generated fluorescence. No further transfers or washing steps are involved. The authors demonstrate the specificity of the method for the detection of ribosomal DNA from Entamoeba histolytica.

L4 ANSWER 29 OF 30 MEDLINE
ACCESSION NUMBER: 97377116 MEDLINE
DOCUMENT NUMBER: 97377116 PubMed ID: 9232865
TITLE: Imaging brain structure and function, infection and gene expression in the body using light.
AUTHOR: Benaron D A; Contag P R; Contag C H
CORPORATE SOURCE: Medical Free Electron Laser Program, Hansen Experimental Physics Laboratory (HEPL), Department of Physics, Stanford University, CA 94034, USA.
CONTRACT NUMBER: N43-NS-6-2313 (NINDS)
N43-NS-6-2315 (NINDS)
RR-00081 (NCRR)
SOURCE: PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY OF LONDON. SERIES B: BIOLOGICAL SCIENCES, (1997 Jun 29) 352 (1354) 755-61. Ref: 18
Journal code: P5Z; 7503623. ISSN: 0962-8436.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 19970908
Last Updated on STN: 19970908
Entered Medline: 19970822

AB Light can be used to probe the function and structure of human tissues. We have been exploring two distinct methods: (i) externally emitting light into tissue and measuring the transmitted light to characterize a region through which the light has passed, and (ii) internally generating light within tissue and using the radiated light as a quantitative homing beacon. The emitted-light approach falls within the domain of spectroscopy, and has allowed for imaging of intracranial haemorrhage in newborns and of brain functions in adults. The generated-light approach is conceptually parallel to positron emission tomography (PET) or nuclear medicine scanning, and has allowed for real-time, non-invasive monitoring and imaging of infection and gene expression in vivo using low-light cameras and ordinary lenses. In this paper, we discuss recent results and speculate on the applications of such techniques.

L4 ANSWER 30 OF 30 MEDLINE
ACCESSION NUMBER: 1998294346 MEDLINE
DOCUMENT NUMBER: 98294346 PubMed ID: 9630890
TITLE: Molecular beacons: probes that fluoresce upon hybridization.
AUTHOR: Tyagi S; Kramer F R
CORPORATE SOURCE: Department of Molecular Genetics, Public Health Research Institute, New York, NY 10016, USA.. sanjay@phri.nyu.edu
CONTRACT NUMBER: AI-37015 (NIAID)
HL-43521 (NHLBI)
SOURCE: NATURE BIOTECHNOLOGY, (1996 Mar) 14 (3) 303-8.
Journal code: CQ3; 9604648. ISSN: 1087-0156.

PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199807
ENTRY DATE: Entered STN: 19980723
Last Updated on STN: 19980723
Entered Medline: 19980714

AB We have developed novel nucleic acid probes that recognize and report the presence of specific nucleic acids in homogeneous solutions. These probes undergo a spontaneous fluorogenic conformational change when they hybridize to their targets. Only perfectly complementary targets elicit this response, as hybridization does not occur when the target contains a mismatched nucleotide or a deletion. The probes are particularly suited for monitoring the synthesis of specific nucleic acids in real time. When used in nucleic acid amplification assays, gene detection is homogeneous and sensitive, and can be carried out in a sealed tube. When introduced into living cells, these probes should enable the origin, movement, and fate of specific mRNAs to be traced.